Mycologia, 95(6), 2003, pp. 1100–1140.

Hypocrea/Trichoderma: species with conidiophore elongations and green conidia

Priscila Chaverri[1]

Pennsylvania State University, Department of Plant Pathology, 301 Buckhout Laboratory, University Park, Pennsylvania 16802

Lisa A. Castlebury

U.S.D.A.-A.R.S., Systematic Botany and Mycology Laboratory, Room 304, B011A, 10300 Baltimore Avenue, Beltsville, Maryland 20705

Barrie E. Overton

Pennsylvania State University, Department of Plant Pathology, 301 Buckhout Laboratory, University Park, Pennsylvania 16802

Gary J. Samuels

U.S.D.A.-A.R.S., Systematic Botany and Mycology Laboratory, Room 304, B011A, 10300 Baltimore Avenue, Beltsville, Maryland 20705

Abstract: Species of *Trichoderma* and *Hypocrea* that have green conidia and sterile or fertile elongations of their conidiophores are described or redescribed and their phylogenetic position explored. The described species include *T. crassum*, *T. fasciculatum*, *T. fertile*, *T. hamatum*, *T. longipile*, *T. oblongisporum*, *T. pubescens*, *T. spirale*, *T. strictipile*, *T. strigosum*, *T. stromaticum*, *T. tomentosum*, *Hypocrea aureoviridis* f. *macrospora*, *H. ceramica*. and *H. semiorbis*. *Trichoderma fasciculatum* originally was described from cultures from ascospores of an unidentified *Hypocrea* specimen; it is considered to be a synonym of *T. strictipile*. The remaining species of *Trichoderma* considered here have not been linked to teleomorphs, and the *Trichoderma* anamorphs of *H. aureoviridis* f. *macrospora* and *H. semiorbis* have not been named. Five new species of *Hypocrea* are described, viz. *H. cremea*, *H. cuneispora*, *H. estonica*, *H. strictipilosa* and *H. surrotunda*. The phylogenetic relationships of these species were inferred based on partial RPB2 and EF-1α DNA sequence data and phenotypic characteristics, including teleomorph, anamorph, colony and growth rates. *Trichoderma crassum* was found to be a sister species to *T. virens*, based on molecular sequences and phenotypic data. *Hypocrea surrotunda* and *H. cremea*, *H. cuneispora* and *T. longipile*, *T. fertile* and *T. oblongisporum*, *T. tomentosum* and *H. atrogelatinosa*, and *T. hamatum* and *T. pubescens*, respectively, were found to be closely related phylogenetically, based on RPB2 and EF-1α gene genealogies. Anamorph and teleomorph phenotype, including conidiophore elongations, phialide morphology, conidial morphology, stroma anatomy and ascospore morphology are not useful predictors of relationships. Despite the shared phenotypic characters of these *Trichoderma* and *Hypocrea* species, they are distributed between two major clades of *Trichoderma/Hypocrea*. Redescriptions and a key to species of *Hypocrea/Trichoderma* with green conidia and conidiophore elongations are presented.

Key words: Ascomycetes, Hypocreaceae, Hypocreales, molecular phylogenetics, RNA polymerase II subunit RPB2, synanamorphs, systematics, teleomorph-anamorph connection, translation elongation factor EF-1α, *Trichoderma* sect. *Pachybasium*

INTRODUCTION

Bissett (1991b) described 12 species of *Trichoderma* Pers. : Fr. and one of *Hypocrea* Fr. that have green conidia and elongations of conidiophores, viz. *T. crassum* Bissett, *T. fasciculatum* Bissett, *T. fertile* Bissett, *T. hamatum* (Bonord.) Bain., *T. longipile* Bissett, *T. oblongisporum* Bissett, *T. pubescens* Bissett, *T. spirale* Bissett, *T. strictipile* Bissett, *T. strigosum* Bissett, *T. tomentosum* Bissett and *H. semiorbis* Berk. The conidiophore elongations in these species can be sterile or can terminate in one or more phialides.

Bissett placed these species in *Trichoderma* section *Pachybasium* (Sacc.) Bissett based on the branching pattern and morphology of the conidiophores (Bissett 1991a, b). This section is now known to be paraphyletic (Kindermann et al 1998) and has been divided into two phylogenetic groups ''A'' and ''B''. Because there is no morphological hiatus between the two groups, for the purposes of the present discussion we will refer to sect. *Pachybasium* in its morphological sense. Most species in sect. *Pachybasium* produce compact conidiogenous pustules with branching in a pyramidal pattern, with or without fertile or sterile conidiophore elongations. Phialides are typi-

Accepted for publication February 26, 2003.

[1] Corresponding author. Current address: U.S.D.A.-A.R.S., Systematic Botany and Mycology Laboratory, Room 304, B011A, 10300 Baltimore Avenue, Beltsville, Maryland 20705. E-mail: priscila@nt.ars-grin.gov

cally short and swollen, produced in verticils of 2–7, and often are crowded. *Hypocrea ceramica* Ellis & Everh., *H. aureoviridis* f. *macrospora* Doi and *Trichoderma stromaticum* Samuels & Pardo-Schultheiss are other species in sect. *Pachybasium* with conidiophore elongations that were not included in Bissett's work.

Most of the members of sect. *Pachybasium* have not been linked to teleomorphs. The anamorph of *H. semiorbis*, a species with colorless ascospores, has been described but not named (Bissett 1991b). The anamorph described by Doi (1972) for *H. aureoviridis* f. *macrospora* falls within the morphological section *Pachybasium*, but there are no available cultures of this taxon. *Hypocrea aureoviridis* f. *macrospora* has green ascospores and an anamorph that has green conidia and a fertile conidiophore elongation (Doi 1972). Cultures from an unnamed species of *Hypocrea* with hyaline ascospores produced *T. stromaticum*, although the ex-type isolate of *T. stromaticum* was not derived from ascospores (Samuels et al 2000). The names *T. fasciculatum* and *T. strictipile* are based on ascospore isolations of unnamed species of *Hypocrea*. The *Hypocrea* specimen from which the *T. fasciculatum* ex-type originated has not been located (CBS, DAOM). The *Hypocrea* specimen from which *T. strictipile* was derived is an undescribed species of *Hypocrea*, with yellowish stromata and green ascospores, and is described here as *H. strictipilosa*. In addition, five unnamed species of *Hypocrea* that have green ascospores and conidiophores with sterile or fertile elongations have been found, mostly in temperate regions.

In the present paper the species of *Trichoderma* that have elongations of conidiophores are more precisely defined through the addition of temperature/growth information and the *Hypocrea* species that have such anamorphs are described or redescribed. Their phylogenetic relationships are explored through partial sequences of the protein-coding genes RNA polymerase II subunit (RPB2) and translation elongation factor (EF-1α). A key to the species of *Hypocrea/Trichoderma* that have elongations of conidiophores and green conidia is included.

MATERIALS AND METHODS

Isolates.—The isolates used in this study are listed in TABLE I. The authors isolated the majority of cultures from *Hypocrea* collections, but others were obtained from Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS), and from Agriculture and Agri-Food Canada, Eastern Cereals and Oilseeds Research Centre, Ottawa, Canada (DAOM). Isolates cultured by P. Chaverri, G. J. Samuels and Barrie E. Overton are preceded by the abbreviations P.C., G.J.S. and B.E.O., respectively. Representative isolates have been deposited in ATCC, CBS and DAOM. Single-ascospore isolations from fresh collections of *Hypocrea* were made on CMD (Difco cornmeal agar + 2% dextrose + distilled water + 1% antibiotic solution (0.2% Sigma Streptomycin Sulfate + 0.2% Sigma Neomycin Sulfate + distilled water)) with the aid of a micromanipulator. These cultures are maintained in CMA (Difco cornmeal agar) slant tubes at 4 C and in liquid nitrogen. Many of the species of *Hypocrea* with green ascospores commonly are identified as *H. gelatinosa* (Tode : Fr.) Fr., *H. flavovirens* Berk. and *H. aureoviridis* Phill. & Plowr. For this reason these species were included in the analysis. Often-cited collectors G. J. Samuels and C. T. Rogerson are abbreviated as G.J.S. and C.T.R., respectively.

Growth and colony characterization.—Growth trials were performed to determine the growth rate and optimum temperature for growth following the protocol of Samuels et al (2002) on PDA (Difco potato-dextrose agar) and synthetic low-nutrient agar (SNA, Nirenberg 1976). The isolates were grown in the dark, and the colony radius was measured at 24, 48, 72 and 96 h at 15, 20, 25, 30 and 35 C. Each growth-rate experiment was repeated three times and the results averaged for each isolate. The time of first appearance of green conidia, the presence of yellow pigmentation of young conidia, the presence of diffusing pigment in the agar, odor and colony appearance also were noted.

Morphological observations.—Morphological observations of the anamorph were taken from cultures grown on CMD in 9 cm diam vented plastic Petri plates in an incubator at 20 C, with alternating 12 h fluorescent light and 12 h darkness within approximately 1 wk. These standard characters were measured: width of phialide base, phialide width at the widest point, phialide length, and length/width ratio (L/W), conidium length, conidium width and length/width ratio (L/W), width of cell from which phialides arise (= metulae, subtending hypha, phialide axis), presence of chlamydospores and chlamydospore width. Measurements of continuous characters were taken from images using the beta 4.0.2 version of Scion Image (Scion Corp., Frederick, Maryland). Colony appearance also was described from CMD at 20 C and PDA at 25 C, with alternating 12 h fluorescent light and 12 h darkness, including formation and shape of tufts or pustules. The presence of chlamydospores was recorded by examining the reverse of a colony grown on CMD after ca 1 wk at 20 C under 12 h darkness and 12 h cool white fluorescent light with 40× objective of a compound microscope. Color terminology was obtained from Kornerup and Wanscher (1978).

The herbarium specimens of *Hypocrea* were rehydrated briefly in 3% KOH. Rehydrated stromata were supported by Tissue-Tek O.C.T. Compound 4583 (Miles Inc., Elkhart, Indiana) and sectioned at a thickness of ca 15 μm with a freezing microtome. These teleomorph characteristics were evaluated: diameter, height, color and shape of the stroma; texture of surface of the stroma; perithecium shape, length and width; reaction to 3% KOH, color, width and KOH reaction of perithecium wall; ostiolar canal length; color and 3% KOH reaction of stroma outer region; shape, diameter and wall thickness of cells of the outer, middle (im-

TABLE I. List of isolates studied, geographic origin and GenBank accession numbers

Species	Isolate	Geographic origin	Substrate	EF-Lα GenBank No.	RPB2 GenBank No.
Hypocrea cf. atrogelatinosa	G.J.S. 95-159	New York, USA	Decorticated wood	AF534603	AF545508
Hypocrea aureoviridis	CBS 245.63	England, UK	*Corylus avellana*	AF534575	AF545509
Hypocrea avellanea	C.T.R. 77-155	Pennsylvania, USA	*Collybia* sp.	AY225857	AF545562
Hypocrea ceramica	G.J.S. 88-70	North Carolina, USA	Decorticated wood	AF534593	AF545510
Hypocrea citrina	CBS 894.85	Belgium	Leaf litter	AY225856	AF545561
Hypocrea cremea	G.J.S. 91-125	New York, USA	Decorticated wood	AF534598	AF545511
Hypocrea cuneispora	G.J.S. 91-93	Virginia, USA	Decorticated wood	AF534600	AF545512
Hypocrea cf. *dichromospora*	G.J.S. 92-123	France	*Phyllostachys bambusoides*	AF534576	AF545513
Hypocrea estonica	G.J.S. 96-129	Estonia	*Hymenochaete tabacina*	AF534604	AF545514
Hypocrea cf. *flavovirens*	P.C. 4	Pennsylvania, USA	Decorticated wood	AF534578	AF545515
Hypocrea gelatinosa	G.J.S. 88-17	France	*Quercus robur*	AF534579	AF545516
Hypocrea lutea	G.J.S. 89-129	New York, USA	Decorticated wood	AF534581	AF545517
Hypocrea megalocitrina	B.E.O. 00-09	North Carolina, USA	*Acer rubrum*	AY225855	AF545563
Hypocrea nigrovirens	G.J.S. 99-64	Limón, Costa Rica	Decorticated wood	AF534582	AF545518
Hypocrea pezizoides	G.J.S. 01-231	Thailand	Wood	AY225859	AF545564
Hypocrea pilulifera	C.B.S. 814-68	Yorkshire, UK	*Betula* sp. wood	AF534583	AF545519
Hypocrea psychrophila	Hy 8	Switzerland	*Rhododendron ferrugineum*	AF534584	AF545520
Hypocrea pulvinata	G.J.S. 98-104	Germany	*Ganoderma* sp.	AY225861	AF545559
Hypocrea rufa	G.J.S. 89-127	North Carolina, USA	Bark	AF534585	AF545521
Hypocrea semiorbis	DAOM 167636	New Zealand	*Nothofagus* sp. wood	AF545568	AF545522
Hypocrea strictipilosa	C.T.R. 77-149	New York, USA	—	AF534589	AF545523
Hypocrea strictipilosa	C.T.R. 78-201	Denmark	Wood	AF534590	AF545524
Hypocrea strictipilosa	G.J.S. 00-170	Russia	*Betula* sp. wood	AF534591	AF545525
Hypocrea strictipilosa	G.J.S. 00-171	Russia	Wood	AF534592	AF545526
Hypocrea strictipilosa	G.J.S. 89-114	Maryland, USA	Decorticated wood	AF534595	AF545527
Hypocrea strictipilosa	G.J.S. 89-115	Maryland, USA	Wood	AF534596	AF545528
Hypocrea strictipilosa	G.J.S. 90-64	New York, USA	*Pinus* sp. wood	AF534597	AF545529
Hypocrea strictipilosa	G.J.S. 91-126	New York, USA	Decorticated wood	AF534599	AF545530
Hypocrea strictipilosa	G.J.S. 94-97	France	*Acer* sp. wood	AF534601	AF545531
Hypocrea strictipilosa	G.J.S. 96-130	Estonia	Wood	AF534605	AF545532
Hypocrea strictipilosa	G.J.S. 96-189	Indiana, USA	*Phellinus ferruginosus*	AF534606	AF545533
Hypocrea strictipilosa	G.J.S. 96-190	Indiana, USA	Bark	AF534607	AF545534
Hypocrea strictipilosa	G.J.S. 98-110	Germany	*Picea* sp.? wood	AF534608	AF545535
Hypocrea strictipilosa	G.J.S. 98-113	Germany	*Picea* sp. wood	AF534609	AF545536
Hypocrea strictipilosa	G.J.S. 98-117	Germany	Decorticated wood	AF534610	AF545537
Hypocrea strictipilosa	G.J.S. 98-91	Pennsylvania, USA	Decorticated wood	AF534612	AF545538
Hypocrea sulphurea	G.J.S. 95-190	Indiana, USA	Bark	AY225858	AF545560
Hypocrea surrotunda	G.J.S. 88-73	Connecticut, USA	Decorticated wood	AF534594	AF545540
Hypomyces stephanomatis	G.J.S. 88-50	North Carolina, USA	*Humaria* sp.	AF534632	AF545566
Nectria cinnabarina	G.J.S. 91-111	Virginia, USA	*Acer* sp. dead branches	AF534633	AF545567

TABLE I. Continued

Species	Isolate	Geographic origin	Substrate	EF-Lα GenBank No.	RPB2 GenBank No.
Trichoderma aggressivum	CBS 100525	United Kingdom	Mushroom compost	AF534614	AF545541
Trichoderma cf. *citrinoviride*	G.J.S. 01-364	New York, USA	Decorticated hardwood	AY225860	AF545565
Trichoderma crassum	DAOM 164916*	Quebec, Canada	*Picea* sp. wood	AF534615	AF545542
Trichoderma crassum	G.J.S. 95-157	New York, USA	Decorticated wood	AF534602	AF545543
Trichoderma fasciculatum	DAOM 167646*	The Netherlands	*Betula* sp. bark	AF534616	AF545544
Trichoderma fertile	DAOM 167070	Canada	Pine forest soil	AF534617	AF545545
Trichoderma fertile	DAOM 167161*	Canada	Soil under *Triticum* sp.	AF534618	AF545546
Trichoderma flavofuscum	DAOM 167652*	Georgia, USA	Soil	AF534619	AF545547
Trichoderma hamatum	DAOM 167057*	Canada	Spruce forest soil	AF534620	AF545548
Trichoderma harzianum	CBS 226.95*	England, UK	Soil	AF534621	AF545549
Trichoderma longipile	DAOM 177227*	Canada	*Ulmus* sp. wood	AF534622	AF545550
Trichoderma oblongisporum	DAOM 167085	Canada	Soil	AF534623	AF545551
Trichoderma pubescens	DAOM 166162*	North Carolina, USA	Forest soil	AF534624	AF545552
Trichoderma spirale	DAOM 183974*	Thailand	Bamboo soil	AF534626	AF545553
Trichoderma strictipile	DAOM 167072	Canada	Maple forest soil	AF534627	AF545554
Trichoderma strictipile	DAOM 172827*	Quebec, Canada	Wood	AF534628	AF545555
Trichoderma strigosum	DAOM 166121*	North Carolina, USA	Forest soil	AF534629	AF545556
Trichoderma stromaticum	P.C. 209	Brazil	*Theobroma cacao* pods	AF534613	AF545539
Trichoderma tomentosum	DAOM 178713A*	Ontario, Canada	*Ulmus* sp. wood	AF534630	AF545557
Trichoderma virens	Gli 39*	USA	Norfolk cultivated soil	AF534631	AF545558

* Ex-type cultures.

mediately below the outer region) and inner region (below perithecia) of the stroma; ascus length and width; distal and proximal part-ascospore length and width. Measurements of continuous characters also were obtained with Scion Image beta 4.0.2. Confidence intervals ($\alpha = 0.05$), minimum and maximum values for the anamorph and teleomorph morphological characters measured were calculated using Systat 8.0 (SPSS Inc., Illinois).

Molecular phylogenetic analysis.—To obtain fresh mycelia for DNA extraction, the isolates listed in TABLE I were grown in potato-dextrose broth (Difco, Detroit, Michigan) in a 5 cm diam Petri plate for 3–5 d. The mycelial mat was dried using clean absorbent paper towels. The entire dried mycelial mat then was placed in a 1.5-mL Eppendorf tube for immediate DNA extraction. Extraction of genomic DNA was done with Puregene™ Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, Minnesota). Fragments of two protein-coding genes were amplified and sequenced: RNA polymerase II subunit (RPB2) and translation elongation factor 1α (EF-1α). The primers used for PCR and sequencing were: fRPB2-5F (5′-GA(T/C)GA(T/C)(A/C)G(A/T)GATCA(T/C)TT(T/C)GG-3′), fRPB2-7cR (5′-CCCAT(A/G)GCTTG(T/C)TT(A/G)CCCAT-3′) (Liu et al 1999); and EF1-983F (5′-GC(C/T)CC(C/T)GG(A/C/T)CA(C/T)GGTGA(C/T)TT(C/T)AT-3′) (Carbone and Kohn 1999), EF1-2218R (5′-ATGAC(A/G)TG(A/G)GC(A/G)AC(A/G)GT(C/T)TG-3′) (S. A. Rehner, pers comm). PCR reactions were set up using these ingredients for each 50 μL reaction: 5 μL of Perkin-Elmer 10× Buffer with $MgCl_2$ (Applied Biosystems, Branchburg, New Jersey), 10 μL of 1 mM dNTPs, 2.5 μL of 10 μM forward primer, 2.5 μL of 10 μM reverse primer, 0.5 μL of Perkin-Elmer AmpliTaq Gold® Taq Polymerase (Applied Biosystems), a maximum of 25 ng/μL of genomic DNA, and double-distilled water to complete a total of 50 μL per reaction. The PCR reactions were placed in a Bio-Rad iCycler thermocycler (Bio-Rad Laboratories, Hercules, California) under the temperature-cycling parameters: Step 1) 10 min at 95 C; Step 2) 40 cycles of 30 s at 94 C, followed by 30 s at 50 C for RPB2 or 55 C for EF-1α, and 1 min at 72 C; and Step 3) 10 min at 72 C. Resulting products were purified with QIAquick® PCR Purification Kit (Qiagen Inc., Valencia, California) and QIAquick® Gel Extraction Kit, when more than one band was amplified. Sequencing was performed at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland) using Perkin-Elmer Big Dye terminators with dITP (Applied Biosystems) and an Applied Biosystems DNA sequencer model 3100. Sequences were edited and assembled using Sequencher 4.1 (Gene Codes, Madison, Wisconsin). Clustal X 1.81 (Thompson et al 1997) was used to align the sequences, and then the alignment was refined by hand. The sequences and alignment were deposited in GenBank (TABLE I) and TreeBase (submission number SN 1244, http://treebase.bio.buffalo.edu/trebase/), respectively.

Phylogenetic analyses were performed using PAUP* 4.0 b10 (Swofford 1999) using *Hypomyces stephanomatis* Rogerson & Samuels and *Nectria cinnabarinna* (Tode : Fr.) Fr. sequences as outgroups. Neighbor-joining analyses (NJ) were performed with the Kimura-2-parameter model. Bootstrap values were calculated from 1000 replicates. In addition, maximum parsimony (MP) also was performed using a heuristic search, with a starting tree obtained via stepwise addition, with 1000 random addition sequences, tree-bisection-reconnection as the branch-swapping algorithm, and MULTREES off. Bootstrap values from 1000 replicates were calculated using a ''fast'' stepwise addition search. A consensus tree was calculated using 50% majority rule. The Incongruence Length Difference Test or Partition Homogeneity Test (PHT) in PAUP* was used to test the congruence among datasets (Cunningham 1997). For this test, parsimony-uninformative characters were excluded, gaps were treated as missing and 500 repetitions were performed. A maximum of 100 trees were saved to conserve computer memory.

RESULTS

Phenotype analysis.—Most of the isolates studied fit the definition of *T. strictipile*; other isolates did not fit any of the described species of *Hypocrea/Trichoderma*. The latter isolates are G.J.S. 91-125, G.J.S. 91–93, G.J.S. 88-73 and G.J.S. 96-129 and therefore are described as new: *H. cremea*, *H. cuneispora*, *H. surrotunda* and *H. estonica*, respectively. In addition, the teleomorph of *T. strictipile* is a new species of *Hypocrea*, *H. strictipilosa*. The specimen of *H. ceramica*, from which isolate G.J.S. 88-70 originated, is indistinguishable from the holotype of this species (Ellis and Everhart 1892). The anamorph of *H. ceramica* described and illustrated in Doi (1966) is indistinguishable from isolate G.J.S. 88-70; the only difference is that in G.J.S. 88-70 conidiophore elongations are fertile or sterile, while the anamorph described in Doi (1966) does not have conidiophore elongations. Unfortunately, Doi's description of the anamorph is based on a Japanese specimen deposited in TNS, which was not available for examination.

The *Trichoderma* members of the morphological section *Pachybasium* are strikingly similar in their morphology. With the exception of *T. crassum*, which is characterized by solitary conidiophores, all the species produce conidiophores in more or less discrete pustules. Phialides of all are broad and short; they tend to be clustered at the ends and along the lengths of broad subtending hyphae that often arise from the base of an elongation that is sterile for a distance to the tip, which remains sterile or produces one or a few ampulliform phialides, depending on the species (TABLE II). Conidia of all are some shade of green, smooth and ellipsoidal to oblong.

Chlamydospores were observed in some species but not in others within about 1 wk on CMD. In most species the chlamydospores are typical of *Hypocrea* in being subglobose and terminal and ca 10 μm diam.

TABLE II. Presence of conidiophore elongations and synanamorphs

Species	Fertile	Sterile	Synanamorph
H. a. f. *macrospora*	+	—	Verticillium-like
H. ceramica	+	+	—
H. cremea	+	—	Verticillium-like
H. cuneispora	+	—	Verticillium-like
H. estonica	+	+	Verticillium-like
H. semiorbis	—	+	—
H. surrotunda	+	+	Verticillium-like
T. crassum	+	+	Penicillium-like
T. fasciculatum	+	—	Verticillium-like
T. fertile	+	—	—
			Verticillium-like
T. hamatum	—	+	—
T. longipile	—	+	—
T. oblongisporum	+	+	Verticillium-like
T. pubescens	—	+	Verticillium-like
T. spirale	—	+	Verticillium-like
T. strictipile	+	+	—
T. strigosum	+	—	—
T. stromaticum	+	—	Verticillium- to
T. tomenstosum	—	+	penicillium-like

Chlamydospores of *T. spirale* are unusual because of the large number that forms within hyphae.

Synanamorphs were observed in 11 species, viz. *T. crassum*, *T. fasciculatum*, *T. hamatum*, *T. pubescens*, *T. spirale*, *T. strictipile*, *T. tomentosum*, *H. cremea*, *H. surrotunda*, *H. estonica* and *H. semiorbis* (TABLE II). The synanamorph is a mononematous conidiophore that arises in the aerial mycelium apart from the pustules. These conidiophores tend to be more or less verticillium- or gliocladium-like and with much longer and more slender phialides than those found in the pustules. Conidia of the synanamorph are held in drops of watery, clear green liquid. Most other synanamorphs found in the other species studied have a sect. *Trichoderma* morphology. There was considerable variation in the conidiophores formed in the ex-type culture of *T. crassum*. This culture produced macronematous conidiophores that formed aggregated pustules with short and wide ampulliform phialides and conidiophore elongations. In the same Petri dish, in areas of effuse conidiation, *T. crassum* produced a gliocladium-like synanamorph with conidia held in drops. The conidia from the pustulate macronematous anamorph were significantly smaller than the ones produced from the gliocladium-like synanamorph. Sequences of RPB2 and EF-1α indicate a very close relationship between *T. crassum* and *T. virens* (Miller et al) Arx. Despite similar colony appearance, and conidiophore and conidial morphology, we have not seen in any isolate of *T. virens* the conidiophore elongations and the macronematous synanamorph that characterize *T. crassum*. Moreover, all five isolates of *T. virens* that we studied previously (Chaverri et al 2001) grow and sporulate well at 35 C, whereas the ex-type culture of *T. crassum* is not able to grow at 35 C (FIG. 1).

The temperature optimum for most species was 25–30 C (FIG. 1), but most of the species had distinctive growth curves. Only *T. flavofuscum* and *T. virens* were able to grow and sporulate at 35 C. In most species there was little difference in growth rate between 25 and 30 C but *H. estonica* was noteworthy for the sharp reduction in growth rate above 25 C. In most species the growth rate was significantly faster on PDA than on SNA, but in *H. cuneispora*, *T. longipile*, *T. hamatum* and *T. crassum* no difference could be detected in the respective growth rates. *Trichoderma virens* and *T. flavofuscum*, which had identical growth rates, were unusual in the genus in growing faster on SNA than on PDA.

The conidiophores and their elongations were as previously described (Bissett 1991b). We did not observe any phenotypic divergence between the ex-type isolates of *T. strictipile* or *T. fasciculatum*. Both had nearly identical growth curves (FIG. 1), colony characteristics or anamorph morphology. The only difference found is that the conidial pustules of the ex-type culture of *T. fasciculatum* are smaller and more loosely organized than those of the ascospore and conidial isolates of *T. strictipile*. The ex-type culture of *T. fasciculatum* was found to be situated amid several isolates of *T. strictipile* in EF-1α and RPB2 sequence analyses.

Molecular phylogenetic analyses.—A total of 903 bp of RPB 2 and 698 bp of EF-1α were included in the analyses. Including only the ingroup taxa, the RPB2 dataset contained 351 polymorphic sites (39%), 72 of them unique. Of the 351 polymorphisms, 54 (15%) are in the 1st codon position, 24 (7%) are in the 2nd codon position and 273 (78%) are in the 3rd codon position. The EF-1α dataset contained 182 polymorphic sites (26%), 53 of them unique. Of the 182 polymorphisms, 32 (18%) are in the 1st codon position, 27 (15%) are in the 2nd codon position and 123 (68%) are in the 3rd codon position.

Molecular phylogenetic analyses show that species of *Hypocrea* and *Trichoderma* with green conidia and conidiophore apical elongations do not form a monophyletic group (FIGS. 2–7). The analyses also confirm that species in morphological sect. *Pachybasium* have multiple evolutionary origins, as suggested earlier by Kindermann et al (1998).

The topologies of the EF-1α and RPB2 gene trees resulting from MP and NJ are similar (FIGS. 2–5). Clades that are highly supported for one gene also

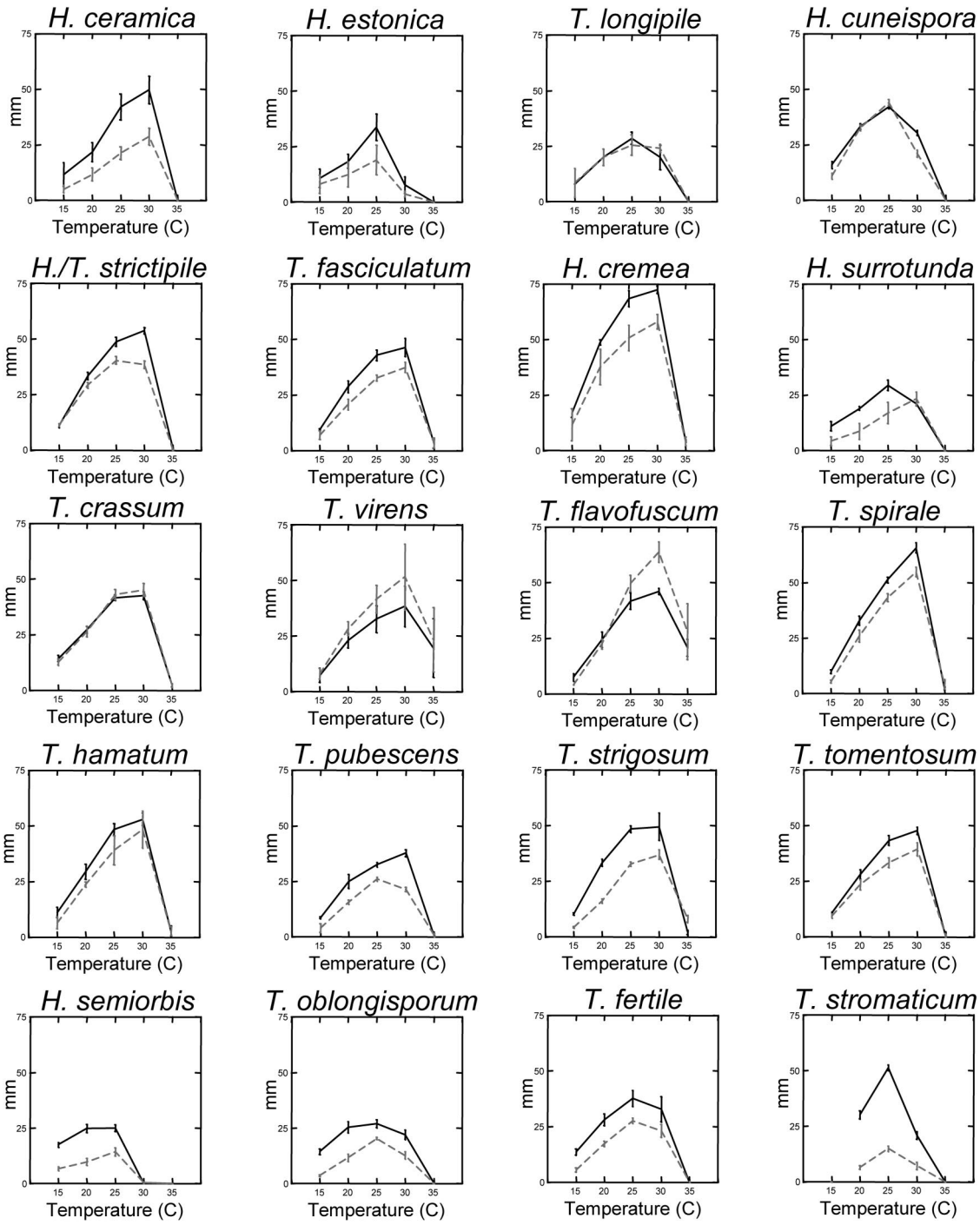
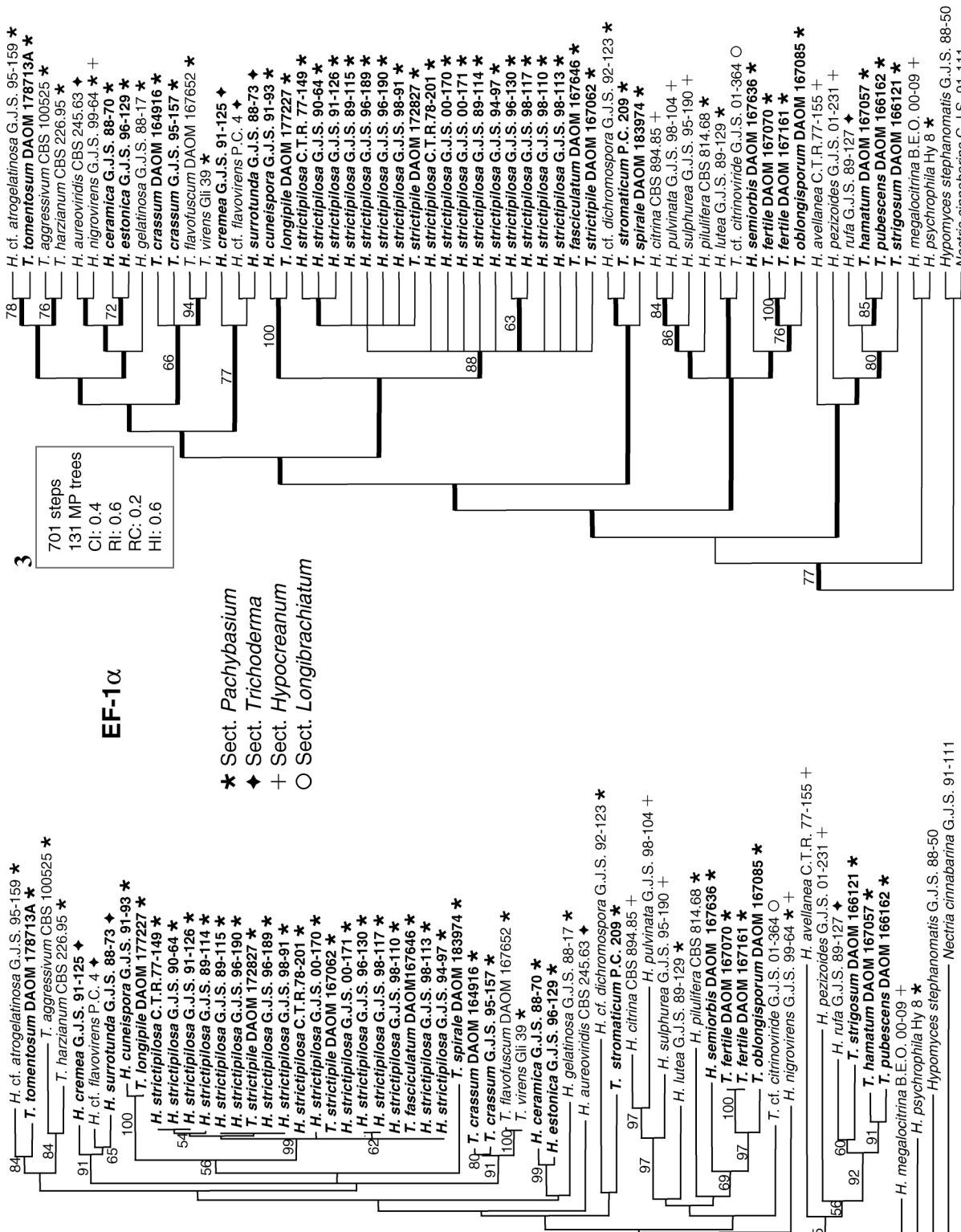
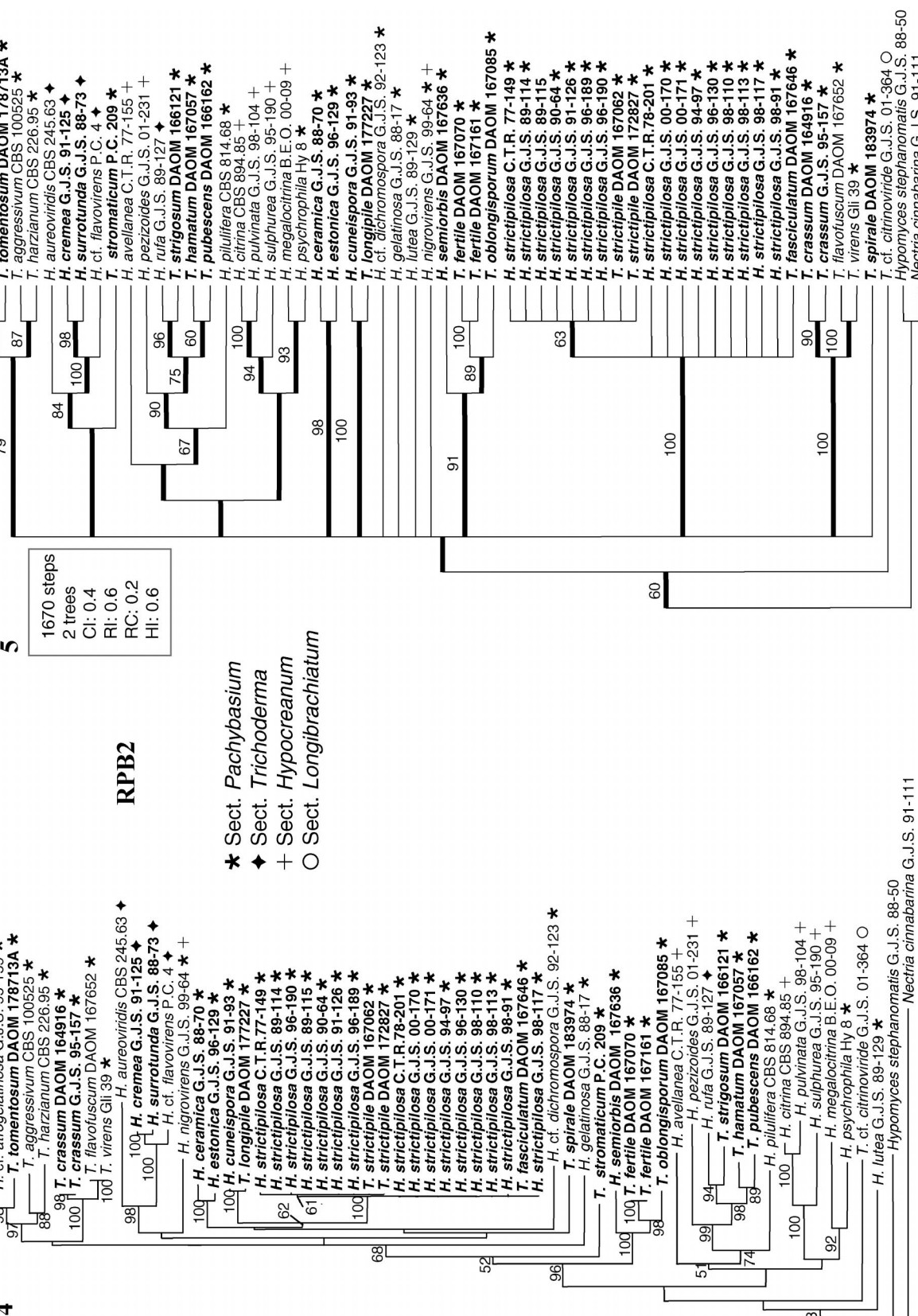


FIG. 1. Radial growth (mm) after 72 h on PDA (—) and SNA (– – –). Tick marks indicate standard error ($\alpha = 0.05$). Graphs are organized according to the phylogenetic and morphological relationship.

are highly supported for the other gene. In general, bootstrap support values are higher for the NJ analyses. The RPB2 gene tree bootstrap values are higher, including the internal nodes, than EF-1α. Even though significant incongruence among data partitions was detected by the PHT (*P*-value = 0.004), we combined the EF-1α and RPB2 partitions based on the "total evidence" approach described in Huelsenbeck et al (1996) to illustrate the phylogeny of relevant species. Most of the disagreements between trees were found at internal nodes and were evident in low bootstrap values for the internal nodes. Bootstrap support values for the combined datasets (FIGS. 6, 7) were higher for terminal and a few internal nodes



FIGS. 2, 3. EF-1α DNA analyses. 2. EF-1α neighbor-joining tree. 3. EF-1α maximum parsimony consensus tree (50% majority rule). Consensus indices >70% are indicated by the thicker lines. Bootstrap values >50% shown at branches. Species with green conidia and conidiophore elongations are indicated in **bold**.



FIGS. 4, 5. RPB2 DNA analyses. 4. RPB2 neighbor-joining tree. 5. RPB2 maximum parsimony consensus tree. Consensus indices >70% are indicated by the thicker lines. Bootstrap values >50% shown at branches. Species with green conidia and conidiophore elongations are indicated in **bold**.

than for the original datasets individually. Most discrepancies were in the phylogenetic position of basal taxa, such as *H. pilulifera*, *T.* cf. *citrinoviride* and *H. lutea*. However, when these taxa were removed from the analyses, the topology and bootstrap supports did not change significantly (analyses not shown). In addition, it is possible that the high incidence of homoplasy contributed to the incongruence between partitions, among other reasons such as different rates of evolution and long-branch attraction (Huelsenbeck et al 1996).

The individual (FIGS. 2–5) and combined genealogies (FIGS. 6, 7) contain several highly supported clades of species producing green conidia and conidiophore elongations. *Trichoderma tomentosum* and *Hypocrea atrogelatinosa* Dingley are supported by bootstrap values >95%. These species are closely related to *T. harzianum* Rifai and *T. aggressivum* Samuels & W. Gams. Two isolates of *T. strictipile* (including the ex-type), the ex-type isolate of *T. fasciculatum* and 16 ascospore isolates from *H. strictipilosa* form a clade supported by 100% bootstrap values. *Trichoderma fasciculatum* has identical RPB2 and EF-1α sequences to the ex-type of *T. strictipile* and other *H. strictipilosa* isolates. All isolates in this group originated from Europe and North America. Most of the specimens of *Trichoderma/Hypocrea* examined with conidiophore elongations belong in this group. *Trichoderma longipile* and *H. cuneispora* form a well-supported clade (100% bootstrap) and appear closely related to the *H. strictipilosa/T. strictipile* clade, although bootstrap values supporting this relationship are low except for the NJ combined analysis (86%). *Trichoderma flavofuscum*, *T. virens* and *T. crassum* form a clade supported by 100% bootstrap values. Within this clade, *T. virens* and *T. flavofuscum* form a clade supported by 100% and have identical RPB2 and EF-1α sequences. Another well-supported clade contains *T. hamatum* ex-type, *T. pubescens* ex-type, *T. strigosum* ex-type and *H. rufa* (bootstrap values >95%). The close relationship between *T. hamatum*, *T. strigosum* and *H. rufa* was discussed in Lieckfeldt et al (1998b). This clade contains species classified in sect. *Trichoderma* and sect. *Pachybasium*. *Trichoderma hamatum* and *T. pubescens*, which are morphologically similar, form a clade that is supported by bootstrap values >60%. The *H. ceramica* and *H. estonica* clade is supported by bootstrap values >90%. The clade that contains *H. flavovirens*, *H. cremea* and *H. surrotunda* is supported by 100% bootstrap value. A close relationship between *T. fertile* and *T. oblongisporum* is supported by bootstrap values >95%. Finally, *T. fertile* isolates and *T. oblongisporum* are closely related to *H. semiorbis*, supported by bootstrap values >95%.

The genealogy of combined EF-1α and RPB2 sequences (FIGS. 6, 7) shows Kindermann's group B (Kindermann et al 1998), which contains most of the species in sect. *Pachybasium* and most of the species studied in this paper. Group B is monophyletic in the NJ tree supported by 88% bootstrap value and paraphyletic in the MP tree. Group B contains at least one group with sect. *Trichoderma* morphology, viz. *H. aureoviridis*, *H. cremea*, *H. surrotunda* and *H.* cf. *flavovirens*. Group A (Kindermann et al 1998), supported here by 100% and 95% bootstrap values in NJ and MP, respectively, contains some species in sect. *Pachybasium*, including the type species, *T. hamatum*. However, it also contains species in sect. *Trichoderma*, such as *H. rufa/T. viride* Pers.

Based on EF-1α and RPB2 gene genealogies, *Hypocrea gelatinosa* s. str. and *H. aureoviridis* s. str. are not closely related to each other or to most of the species of *Hypocrea/Trichoderma* with green conidia and conidiophore elongations considered in this study. *Hypocrea aureoviridis* is closely related to the group that contains *H. cremea*, *H. surrotunda* and *H.* cf. *flavovirens* in RPB2 phylogeny. *Hypocrea* cf. *flavovirens* is closely related to *H. cremea* and *H. surrotunda* and together form a clade supported by 100% bootstrap value.

DISCUSSION

The combined RPB2 and EF-1α phylogeny presented in this study dealing with 38 species, demonstrates once more that morphology, such as conidiophore elongations and synanamorphs, is not always useful in resolving evolutionary relationships in *Hypocrea/Trichoderma*.

Molecular phylogenetic analyses show that elongations of the conidiophore and the pachybasium-like morphology of conidiophores have evolved or have been lost multiple times within *Hypocrea/Trichoderma*. This has been shown previously by Kindermann et al (1998) who, using ITS 1 sequences, divided the morphological section *Pachybasium* into two groups A and B, and group A was further subdivided into A1 and A2. The type species of sect. *Pachybasium*, *T. hamatum*, clustered in A2 along with *T. viride*, the type species of the genus, *T. pubescens* and *T. strigosum*. Clade A1 contains *H. pilulifera*. Sixteen of the species included in our study, including the new *Hypocrea* species, cluster in group B. However, in the maximum-parsimony analysis group B is paraphyletic. Clade A2 contains *T. hamatum*, *T. pubescens*, *T. strigosum*, and clade A1 contains *H. pilulifera*. Once again, sect. *Pachybasium* is shown to be polyphyletic, but we retain this name as a descriptor of the mor-

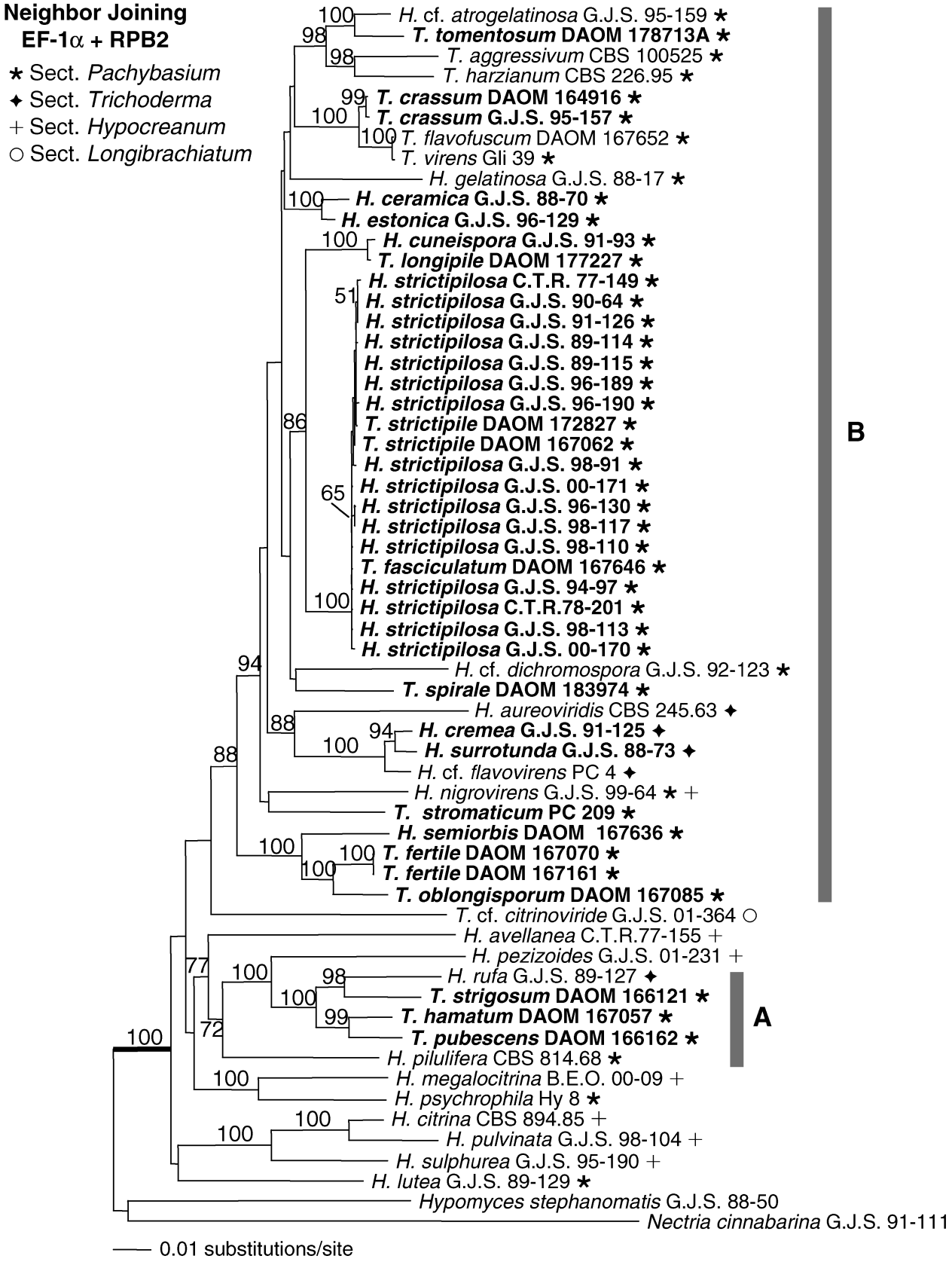


FIG. 6. Combined RPB2 and EF-1α neighbor-joining tree. Bootstrap values shown at each branch. Species with green conidia and conidiophore elongations are indicated in **bold**.

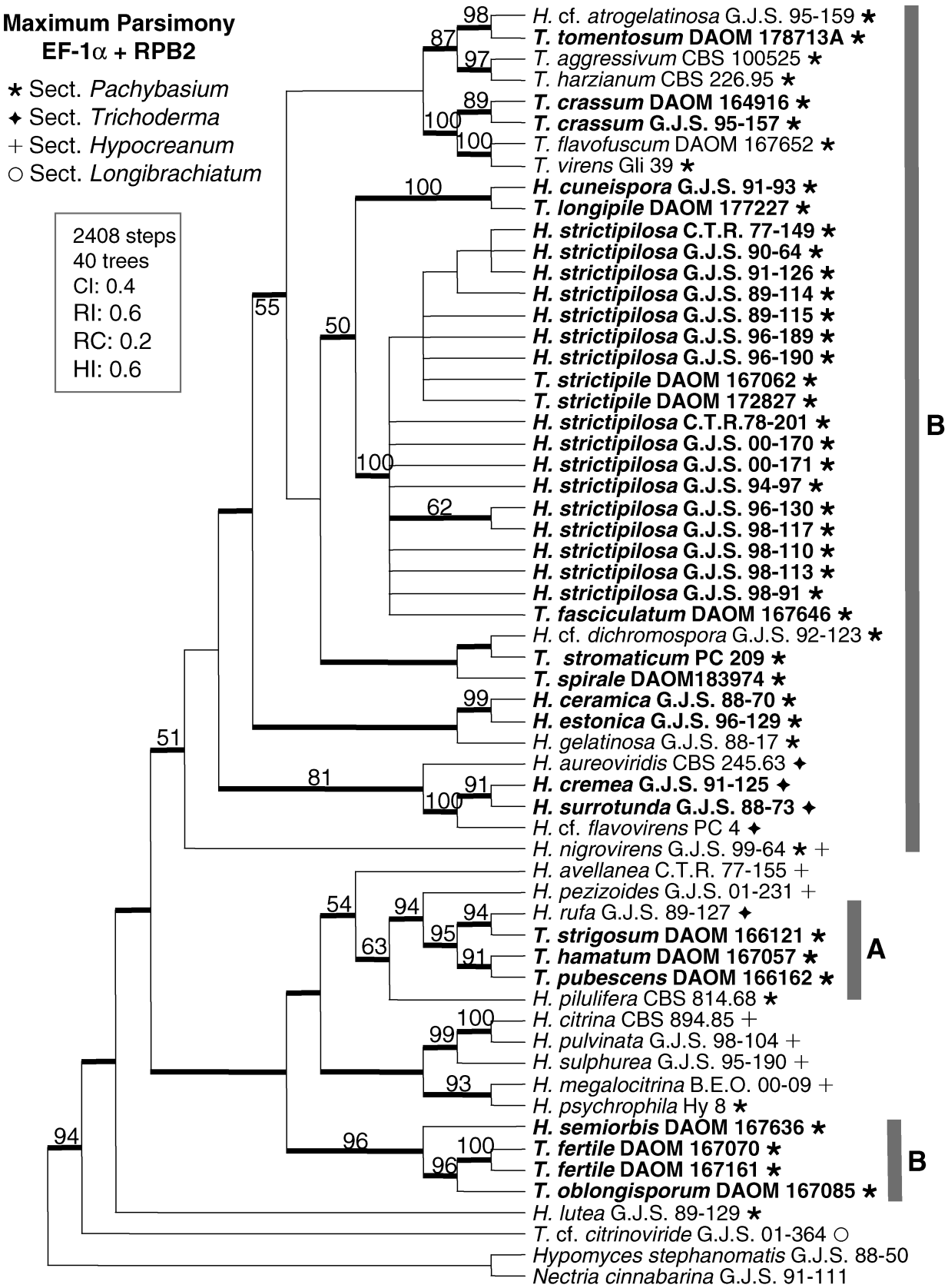


FIG. 7. Combined RPB2 and EF-1α maximum parsimony consensus tree. Bootstrap values >50% shown above branches. Consensus indices >70% are indicated by the thicker lines. Bootstrap values >50% shown at branches. Species with green conidia and conidiophore elongations are indicated in **bold**.

phology that is shared by all members of this artificial section.

Teleomorph morphology in *Hypocrea* is not always useful to distinguish species. *Hypocrea cremea*, *H. estonica*, *H. surrotunda*, *H. strictipilosa*, *H. aureoviridis* f. *macrospora* and *H. cuneispora* have small, yellowish pulvinate stromata. *Hypocrea strictipilosa*, *H. a.* f. *macrospora* and *H. cuneispora*, all have larger ascospores. They can be distinguished primarily by the anamorph and, in the case of *H. cuneispora*, by the ''scaly'' appearance of the surface of the stroma. In contrast, *H. ceramica* has a reddish-brown stroma that is very distinctive and spinulose ascospores. *Hypocrea semiorbis* and the teleomorph of *T. stromaticum* both have colorless ascospores. The teleomorph of *H. a.* f. *macrospora* resembles *H. aureoviridis* sensu stricto; however the anamorph of *H. a.* f. *macrospora* morphologically is distinct from that of *H. aureoviridis* (Lieckfeldt et al 2001).

Trichoderma strictipile was described on the basis of an ascospore isolate from an unnamed *Hypocrea* species, which is described here as *H. strictipilosa*. This species is common in eastern North America and Northern Europe, as far east as the Moscow region of Russia. *Trichoderma fasciculatum* also is derived from a *Hypocrea* specimen, but we could not locate that collection in CBS or DAOM. The ex-type and only known isolate of *T. fasciculatum* is situated amid *T. strictipile*. In its morphological and molecular characters it is indistinguishable from *T. strictipile*. Bissett (1991b) considered the two species to be closely related and distinguished them only through the morphology of the conidiophore elongation, straight in *T. fasciculatum* and flexuous in *T. strictipile*. The isolates that we studied can have both types of conidiophore elongations in the same isolate. Therefore, we conclude that *T. fasciculatum* is a synonym of *T. strictipile*.

Sequence data indicate a close relationship between *H. cuneispora* and *T. longipile*, but each is represented by only a single culture and the two anamorphs are so different in their morphology, growth rates and colony characters that we cannot consider them to be the same species. A close relationship between these species and *H. strictipilosa*/*Trichoderma strictipile* also is indicated.

The RPB2 and EF-1α gene genealogies have revealed phylogenetic ''sister species'' that differ slightly in sequence and share phenotypic characters. For example, the anamorphs of *H. cremea* and *H. surrotunda* are almost indistinguishable. The main differences are found in the growth rate on PDA, an average difference of ca 0.5 μm in conidial length and in the morphology of the stroma. Another example is the similarity between *T. hamatum* and *T. pubescens*. Bissett (1991b) had mentioned that these two species were closely related but that he could distinguish them on the morphology of the conidiophore elongations, a difference that we found difficult to see. However, it was found that the growth rate on PDA is significantly faster in *T. hamatum*. Small but significant differences in the length of the phialides and conidia between the two species also were observed. We have not ruled out the possibility that *T. pubescens* is a synonym of *T. hamatum*, but to make a definite conclusion more isolates of both species need to be studied. *Trichoderma fertile* and *T. spirale* also are difficult to distinguish morphologically; however RPB2 and EF-1α gene genealogies show that they are not closely related. We could distinguish *T. fertile* from *T. spirale* by the slightly smaller conidia, slightly larger phialides, fertile conidiophore apical elongations and slower growth rate on PDA. These examples illustrate the necessity of using a combination of molecular and phenotypic characters to delimit species in *Hypocrea*/*Trichoderma*.

Phylogenetic reconstruction in *Hypocrea*/*Trichoderma* has relied most heavily on sequence analysis of the ITS region of rDNA (Dodd et al 2000, Kindermann et al 1998, Kuhls et al 1997, Lieckfeldt et al 1998a). However, results of these analyses have not sufficiently resolved relationships of species or separated species within closely related complexes. In addition, some of the isolates studied in this paper considered to be the same species have very different ITS sequences (results not shown). Consequently, the ITS sequence data produce a tree topology that is very distinct from RPB2 and EF-1α phylogenies. For example, we observed that certain isolates of *T. strictipile* and *T. crassum*, respectively, possessed divergent ITS 1 sequences, placing them in at least three different positions in the tree. In contrast, RPB2 and EF 1-α tree topologies were highly congruent and *T. strictipile* and *T. crassum* isolates were monophyletic. Although it is not clear why this happened in our study, the presence of different non-orthologous ITS types has been shown previously for *Fusarium* spp. (O'Donnell and Cigelnik 1997) and a similar situation might be occurring in *Trichoderma*.

Until now, there were 13 described species of *Trichoderma* with green conidia and conidiophore apical elongations. In this paper, five more unnamed species were found to be distinct from the named species and therefore are described as new.

KEY TO SPECIES OF *HYPOCREA* AND *TRICHODERMA* WITH GREEN CONIDIA AND CONIDIOPHORE ELONGATIONS

1. Growing on PDA at 30 C after 72 h 2
1. Not growing on PDA at 30 C; teleomorph with hyaline

ascospores; known only from Australia/New Zealand . . . . . 12. *H. semiorbis*
2. Stromatic pustules formed of vesicular to pseudoparenchymatous cells arranged in chains that tend to branch dichotomously near the surface of the pustule; known only from *Theobroma cacao* . . . . . 16. *T. stromaticum*
2. Pustules not stromatic 3
3. Conidia with L/W ≥ 1.6 4
3. Conidia with L/W < 1.6 7
4. Phialides 7.2–17.0 × 1.5–2.5 μm, L/W > 2.0 . . . . . 1. *H. aureoviridis* f. *macrospora*
4. Phialides < 7.2 μm long, L/W < 2.0 5
5. Phialides 4.2–6.0 × 3.0–4.5 μm; conidia 4.0–5.7 × 2.0–3.0 μm (L/W 1.6–2.0) 6
5. Phialides 6.0–7.2 × 3.7–4.0 μm (L/W 1.5–2.0); conidia 5.5–6.0 × 3.2–3.5 μm (L/W 1.6–1.8) (phialides from conidiophore elongation apices 9.7–15.7 × 2.2–3.7 μm, L/W 3.0–5.5) . . . . . 5. *H. cuneispora*
6. Conidia with L/W ca 2.0, 4.0–5.7 × 2.0–3.0 μm; phialides 4.2–6.0 × 3.0–4.5 μm; conidiophore elongations flexuous and frequently branched . . . . . 9. *T. longipile*
6. Conidia with L/W 1.6, 4.5–4.7 × 2.7–3.0 μm; phialides 5.5–6.0 × 3.7–4.0 μm; conidiophore elongations relatively straight, unbranched . . . . . 10. *T. oblongisporum*
7. Phialides ≥ 8.2 μm long 8
7. Phialides < 8.2 μm long 10
8. Phialides from all kinds of conidiophores 8.2–10.5 μm long 9
8. Phialides from gliocladium-like synanamorph 12.5–17.0 × 4.0–5.0 μm (L/W 3.0–3.8); conidia 5.5–6.5 × 4.2–5.0 μm (L/W 1.2–1.4) (macronematous pustulate anamorph sometimes present with phialides 4.5–9.5 × 3.0–4.2 μm, conidia 3.7–5.2 × 2.5–3.7 μm) . . . . 3. *T. crassum*
9. Phialides 8.2–9.5 × 3.5–4.0 μm (L/W 2.2–2.6), conidia 4.5–5.0 × 3.7–4.0 μm (L/W 1.2–1.3); radial growth slow, on PDA after 72 h at 25 C: 26–32 mm and at 30 C: 20–22 mm . . . . . 17. *H. surrotunda*
9. Phialides 9.0–10.5 × 3.2–3.5 μm (L/W 2.7–3.3); conidia 4.0–4.5 × 3.5–3.7 μm (L/W 1.1–1.2); radial growth fast, on PDA after 72 h at 25 C: 63–72 mm and at 30 C: 70–74 mm . . . . . 4. *H. cremea*
10. Conidia with L/W 1.1–1.3 11
10. Conidia with L/W ≥ 1.3 13
11. Conidia 3.3–3.7 × 2.7–3.0, L/W 1.1–1.3; stroma reddish-brown . . . . . 2. *H. ceramica*
11. Conidia ≥ 4.0 μm long; stroma yellowish 12
12. Conidia 4.0–4.2 × 3.2–3.5, L/W 1.2–1.3; phialides from synanamorph 14.7–18.2 × 2.5–3.0, L/W 5.4–7.9 . . . . . 6. *H. estonica*
12. Conidia 4.5–4.7 × 3.5–3.7, L/W 1.3; phialides from synanamorph 13.0–14.5 × 3.5–3.7, L/W 3.8–4.3 . . . . . 14. *H. strictipilosa*/*T. strictipile*
13. Conidia 3.2–3.5 × 2.2–2.5 μm (L/W 1.4); phialides 4.5–5.0 × 3.0–3.2 μm (L/W 1.5–1.6) (phialides from apical conidiophore elongations 8.5–10.0 × 2.5–2.7 μm, L/W 3.2–3.8, conidia 3.5–3.7 × 2.5–2.7 μm, L/W 1.3–1.4) . . . . . 18. *T. tomentosum*
13. Conidia > 3.6 μm long, L/W > 1.4 14
14. Conidiophore elongations branched, with long branches, sterile 15
14. Conidiophore elongations mostly unbranched, and when branched then branches short and fertile 16
15. Conidiophore elongations thin, flexuous, branching; phialides 5.5–6.0 × 3.5–3.7 μm (L/W 1.5–1.7); conidia 4.0–4.2 × 2.7–3.0 μm, L/W 1.5–1.6; colonies on PDA at 25 C after 72 h 32–33 mm radius . . . . . 11. *T. pubescens*
15. Conidiophore elongations, generally straight, branching; phialides 5.2–7.2 × 3.2–4.2 μm (L/W 1.6–1.8); conidia 4.2–5.0 × 2.7–3.0 μm, L/W 1.5–1.6; colonies on PDA at 25 C after 72 h 45–52 mm radius . . . . . 8. *T. hamatum*
16. Gliocladium-like synanamorph always present. Conidiophore apical elongations straight, unbranched, generally sterile, when fertile with one or two phialides; phialides 4.5–8.2 × 3.0–4.2 μm; conidia 3.7–5.2 × 2.5–3.7 μm (L/W 1.4) . . . . . 3. *T. crassum*
16. Gliocladium-like synanamorph absent 17
17. Conidia 3.5–4.5 × 2.0–3.0 μm (L/W 1.5–1.6), subcylindrical oblong; phialides 5.0–8.0 × 2.7–3.7 μm; conidiophore elongations straight, rarely undulate, usually branched irregularly just above the fertile part, usually with a single phialide at the tip . . . . . 15. *T. strigosum*
17. Conidia 3.5–4.5 × 2.5–3.0 μm (L/W 1.4–1.5), ellipsoidal; phialides 4.5–6.5 × 3.0–4.7 μm; conidiophore elongations generally unbranched, when branched then with a short branch near the tip of the elongation 18
18. Conidia 3.5–4.0 × 2.5–3.0 μm; phialides 5.2–6.5 × 3.5–4.7 μm (L/W 1.3–2.0); conidiophore elongations straight, generally with one or two short, fertile apical branches; colonies on PDA at 30 C after 72 h 18–55 mm; no known synanamorph . . . . . 7. *T. fertile*
18. Conidia 3.5–4.5 × 2.5–3.0 μm; phialides 4.5–6.5 × 3.0–3.7 μm (L/W 1.3–2.0); conidiophore elongations flexuous, unbranched, sterile; colonies on PDA at 30 C after 72 h 60–70 mm radius; synanamorph formed in aerial mycelium . . . . . 13. *T. spirale*

SPECIES DESCRIPTIONS

1. *Hypocrea aureoviridis* f. *macrospora* Doi, Bull. Natl. Sci. Mus. Tokyo 15: 728. 1972.

Notes.—We examined the isotype and two paratype specimens of *H. aureoviridis* f. *macrospora* (NY). Although an anamorph was described for this taxon, none of the cultures is available. This taxon resembles *H. cuneispora* and *H. strictipilosa* in the large ascospores, anamorph with fertile conidiophore apical elongations and yellowish stroma. The *forma* differs from *H. strictipilosa* in conidial length/width ratio, respectively ca 1.7 and 1.2–1.3. *Hypocrea aureoviridis* f. *macrospora* can be distinguished from *H. cuneispora* by the slightly longer and more slender phialides and slightly smaller ascospores. In addition, *H. a.* f. *macrospora* seems to be restricted to Japan and the *H. cuneispora* holotype is from the U.S.A. The teleomorph of *H. a.* f. *macrospora* resembles *H. aureoviridis* sensu stricto; however, the anamorph of *H. a.* f. *macrospora* is morphologically distinct from that of *H. aureoviridis*, *T. aureoviride* Rifai (Lieckfeldt et al 2001). Therefore, we doubt that *H. a.* f. *macrospora* is a *forma* of *H. aureoviridis* s. str., but in the absence of additional fresh material we do not propose to change its status.

Specimens examined.—JAPAN. GUNMA PREF.: Hontani, Naruhara, Ueno-Mura, Tano-Gun, on decorticated wood, 5 Aug 1967, Y. Doi (TNS.D-332 = TNS-F-191616, PARATYPE: NY). NAGANO PREF.: Near Asama-Sanso, the foot of Mount Asama, Komoro City, on decorticated wood, 13 Aug 1967, Y. Doi (TNS.D-333 = TNS-F-223414, PARATYPE: NY), on decorticated wood, 11 Aug 1966, Y. Doi (TNS.D-148 = TNS-F-191611, ISOTYPE: NY).

2. *Hypocrea ceramica* Ellis et Everh., North Amer. Pyrenomyc. p. 85. 1892 FIGS. 8–15, 140, 148, 168

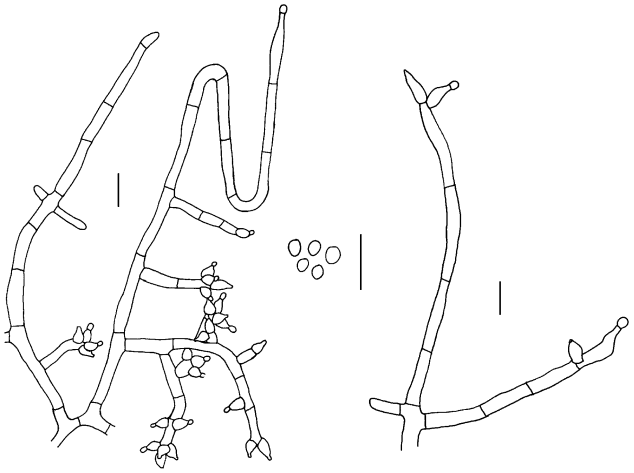


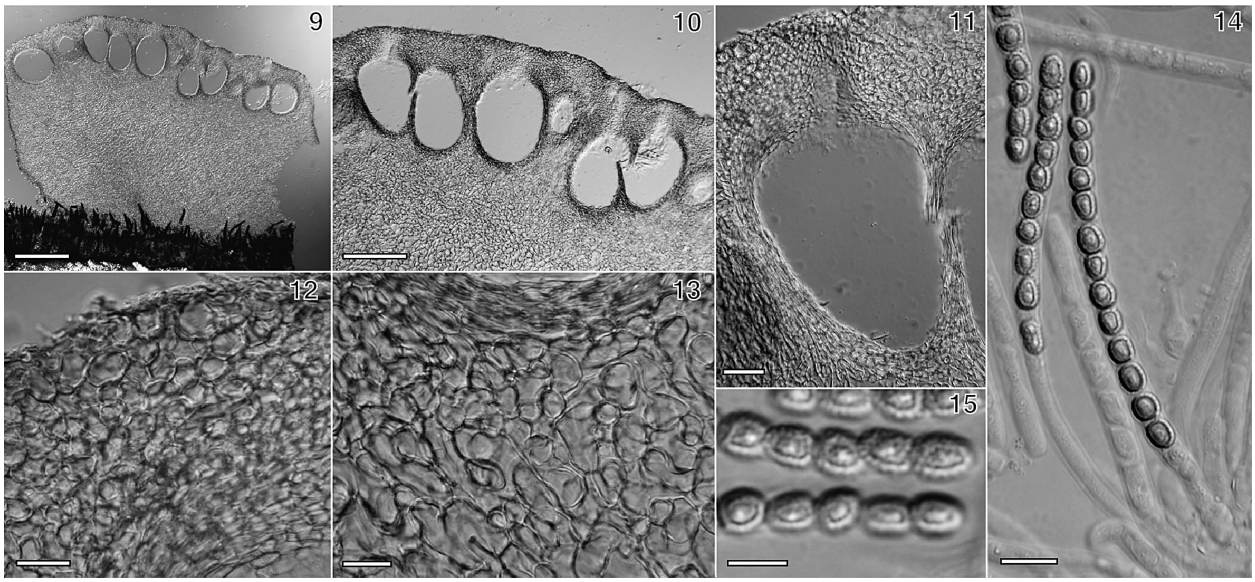
FIG. 8. *Hypocrea ceramica* anamorph (isolate G.J.S. 88-70). Scale bars = 10 μm.

≡ *Chromocrea ceramica* (Ellis et Everh.) Seaver, Mycologia 2: 59. 1910.

Anamorph.—*Trichoderma* sp.

Stromata scattered, pulvinate, nearly circular in outline, (0.6–)0.8–1.7(–2.1) mm diam (n = 30), 0.8–1.0 mm high (n = 10), broadly attached, surface smooth, with perithecial protuberances, reddish brown, not changing color in KOH, ostiolar openings obvious due to the discharged green ascospores. Tissue of the stroma of *textura angularis*. Stroma surface cells (3.4–)4.8–6.3(–8.3) μm diam (n = 30), walls (0.5–)0.6–0.8(–0.9) μm thick (n = 30). Tissue immediately below the surface layer and between perithecia of *textura angularis,* cells (3.1–)5.0–6.4(–7.8) μm diam (n = 25), walls (0.3–)0.4–0.5(–0.6) μm thick (n = 20). Internal tissue below the perithecia of *textura angularis,* cells (5.3–)6.7–8.0(–10.8) μm diam (n = 30), walls (0.5–)0.6–0.7(–1.1) μm thick (n = 20). Perithecia immersed in the stroma, generally closely aggregated, subglobose, 195–260 μm high, 112–230 μm wide (n = 10), wall composed of compacted cells, turning brownish in KOH, (9–)13–15 (–17) μm thick (n = 20), ostiolar canal 40–90 μm long (n = 10). Asci cylindrical, (50–)68–95(–96) × (3.2–)4.5–5.5(–7.7) μm (n = 35), ascospores uniseriate. Part-ascospores green, spinulose, dimorphic, distal part globose to subglobose, (3.5–)3.9–4.7(–5.8) × (2.9–)3.4–4.2(–4.3) μm, proximal part cuneiform, (3.4–)4.2–5.2(–6.2) × 1(2.9–)3.1–3.7(–4.0) μm (n = 60).

Colonies on CMD at 20 C after ca 10 d flat, with discrete tufts 2–3 mm diam (n = 3) forming in concentric rings from the point of inoculum outward, no odor, no pigmentation of agar. Conidiophores irregularly branched, phialides ampulliform, broader in the middle, and constricted at the tip, formed in whorls of (1–) 3 (–4), (5.0–)6.5–8.0(–13.0) μm long, (2.5–)3.5–4.0(–4.5) μm at the widest point, (2.0–)2.7–3.1(–3.7) μm at the base, L/W (1.2–)1.7–2.2(–4.4) (n = 30), elongations of the conidiophore rare, fertile or sterile, generally terminating in one phialide; subtending hyphae cylindrical, (3.0–)3.5–4.0(–4.3) μm wide (n = 15). Conidia green, smooth, ellipsoidal, (2.8–)3.3–3.7(–4.2) × (2.1–)2.7–3.0(–3.1)



FIGS. 9–15. *Hypocrea ceramica* teleomorph (G.J.S. 88-70). 9, 10. Section of stroma. 11. Perithecium. 12. Tissue of surface of stroma. 13. Internal tissue below perithecia. 14. Asci and ascospores. 15. Ascospore ornamentation. Scale bars: 9 = 300 μm, 10 = 200 μm, 11 = 50 μm, 12, 14 = 10 μm, 13 = 15 μm, 15 = 5 μm.

μm, L/W (0.9–)1.1–1.3(–1.7) (n = 20). No chlamydospores observed.

Colonies on PDA at 25 C after ca 2 wk flat, aggregated pustules forming from the point of inoculum outward, young conidia pale yellow, no pigmentation of agar, and no distinctive odor. Colony radius after 3 d on PDA at 15 C: 5–19 mm, 20 C: 16–27 mm, 25 C: 35–50 mm, 30 C: 41–57 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 3–7 mm, 20 C: 9–16 mm, 25 C: 17–24 mm, 30 C: 23–32 mm, and 35 C: 0 mm (n = 3).

Habitat.—Decorticated wood.

Known distribution.—United States and Japan.

Holotype.—UNITED STATES. CONNECTICUT: West Haven, on decaying limb of *Juniperus*, Nov 1888, R. Thaxter (NY).

Additional specimen examined.—UNITED STATES. NORTH CAROLINA: Transylvania County, North of Brevard, Pisgah National Forest, fish hatchery, on decorticated wood, 29 Sep 1988, K. F. Rodrigues, C.T.R., G.J.S., E. Parmasto, R. H. Petersen (NY, culture: G.J.S. 88–70).

Notes.—*Hypocrea ceramica* can be distinguished by the reddish brown to brick-red stroma, a color that is rare in *Hypocrea*, and the small conidia and short phialides. The conidiophore elongation is a rare trait in this species. *Hypocrea ceramica* phylogenetically is closely related to *H. estonica* based on RPB2 and EF-1α molecular sequence data. Both species have small conidia compared to the other species studied in this paper.

3. *Trichoderma crassum* Bissett, Can. J. Bot. 69: 2376.1991. FIGS. 16–24, 149, 169

Colonies on CMD at 20 C after ca 1 wk flat, with fasciculate pustules formed near the point of inoculum. Conidiophores on CMD at 20 C after ca 1 wk, in minute pustules or effuse, branching irregularly, with clusters of short ampulliform phialides. Phialides mainly arising in crowded whorls of 2–5, less frequently in pairs, straight, short, ampulliform, base constricted, swollen in the middle, attenuate at the tip, 4.4–9.5 × 3.0–4.2 μm, arising from a cell (3.2–)4.6–5.0(–5.7) μm wide (n = 20). Conidiophore elongations sometimes present, fertile, rarely sterile. Synanamorph conidiophores arising singly in the aerial mycelium, conidiophores (6.0–)20.0–28.5(–49.0) × (4.5–)5.2–6.0(–8.0) μm (n = 15) with each branch terminating in a penicillus of (2–)3–4 closely appressed phialides; phialides (8.5–)13.5–15.7(–28.0) μm long, (3.3–)4.3–4.6(–5.7) μm at the widest point, (2.2–)3.0–3.3(–4.8) μm at the base, L/W (1.8–)3.1–3.6(–7.3) (n = 60). Long unbranched conidiophores with one or two phialides at the tip sometimes present in the aerial mycelia, ca 200–300 μm long (n = 10). Phialides arising from unbranched long conidiophores 24.8–29.3 μm long, 2.9–3.0 μm at the widest point, 3.0–3.5 μm at the base, L/W 8.2–10.0 (n = 30). Conidia green, smooth, broadly ellipsoidal to obovoid, 3.7–5.3 × 2.6–3.7 μm, L/W ca 1.4. Conidia from gliocladium-like synanamorph, in areas of effuse conidiation, (5.1–)5.9–6.4(–8.0) × (3.9–)4.7–4.9(–5.7) μm, L/W ca 1.3 (n = 60). Chlamydospores present in some isolates, terminal and intercalary, subglobose to ellipsoidal, 6–17 μm diam (n = 15).

Colonies on PDA at 25 C after ca 1 wk slightly cottony, conidiation effuse or aggregated in minute flat pustules, <1 mm diam, formed around the point of inoculum, diffusing yellow pigment sometimes found, no distinctive odor detected. Colony radius after 3 d on PDA at 15 C: 10–20 mm, 20 C: 22–33 mm, 25 C: 35–45 mm, 30 C: 36–49 mm, and 35 C: 0–5 mm (n = 9). Colony radius after 3 d on SNA at 15 C: 8–19 mm, 20 C: 17–35 mm, 25 C: 34–53 mm, 30 C: 33–57 mm, and 35 C: 0–6 mm (n = 9).

Habitat.—Wood and soil.

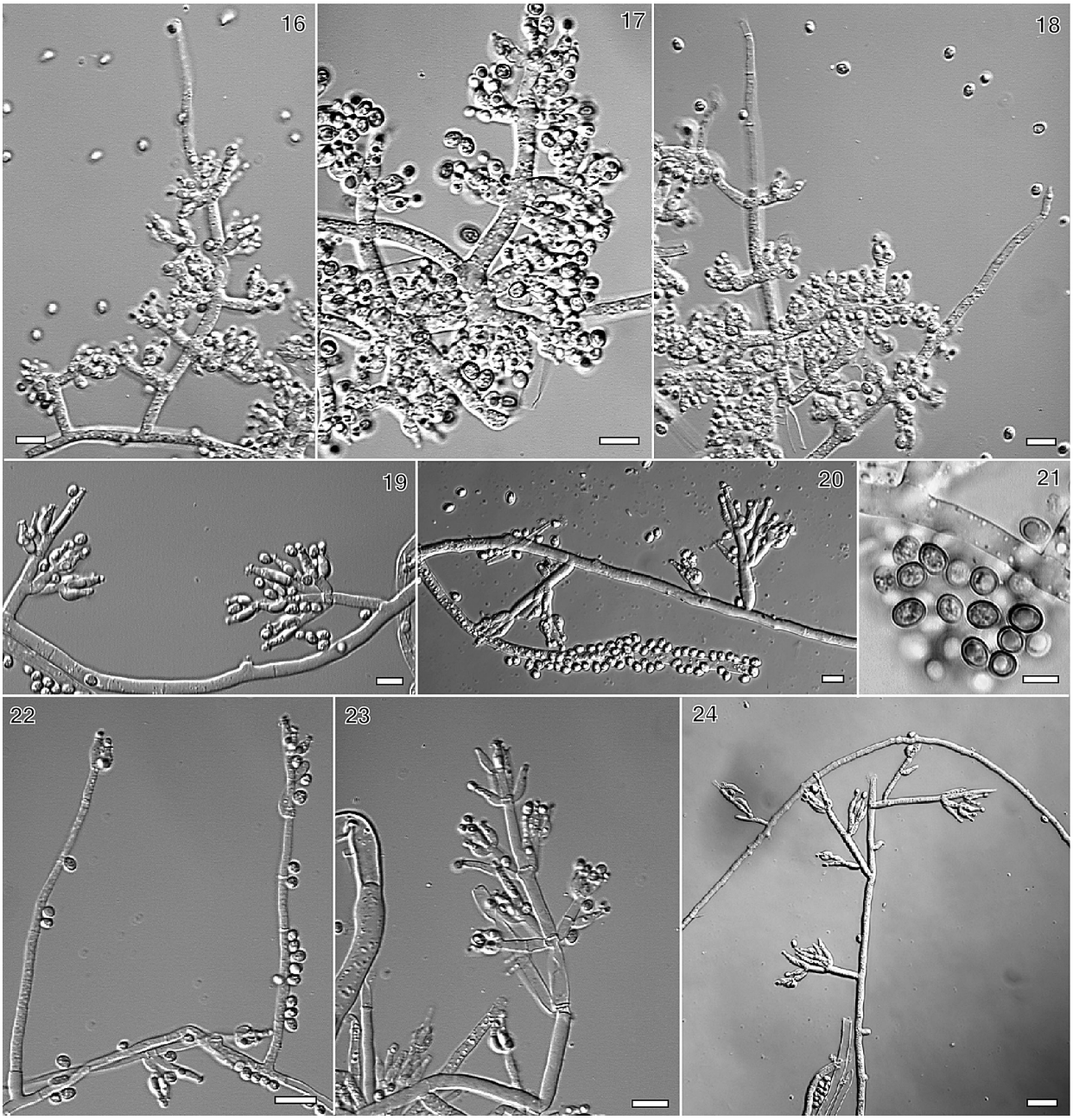
Known distribution.—North America, New Zealand.

Specimens examined.—CANADA. QUEBEC: Lacolle, from Norway spruce plantation soil, May 1977, P. Widden (culture: DAOM 164916, ex-type). NEW ZEALAND. Tokoroa, Maiden Tram Rds., from soil under *Pinus radiata*, 24 Sep 1997, A. Chee (culture: G.J.S. 98-179). UNITED STATES. NEW YORK: Tompkins County, Ringwood-Lloyd Preserve, on decorticated wood, 13 Sep 1995, K. T. Hodge (culture: G.J.S. 95-157, as *Hypocrea* sp., specimen lost).

Notes.—The ex-type isolate of *T. crassum* exhibits two types of anamorphs: one pustulate with macronematous conidiophores that has clusters of short phialides that form aggregated, irregular pustules on PDA and CMD, and another anamorph that has gliocladium-like conidiophores that do not form pustules and are rather spread out through the plate. Other cultures examined did not exhibit the macronematous synanamorph or it was rare. One of the isolates examined, G.J.S. 95-157, originated from ascospores from an unidentified species of *Hypocrea* with green ascospores. Unfortunately this specimen has been lost and therefore the teleomorph of *T. crassum* could not be described. *Trichoderma crassum* phylogenetically is closely related to *T. virens* based on RPB2 and EF-1α molecular data. The gliocladium-like synanamorph of *T. crassum* is almost indistinguishable from that of *T. virens*. *Trichoderma crassum* can be separated easily from *T. virens* by the inability of the former species to grow at 35 C.

4. *Hypocrea cremea* Chaverri et Samuels, sp. nov. FIGS. 25–29, 141, 150, 170

Stromata albolutea, modice perlucida, (0.8–)0.9–1.2(–1.5) mm diam. Asci cylindrici, (99–)104–110(–120) × (4.7–



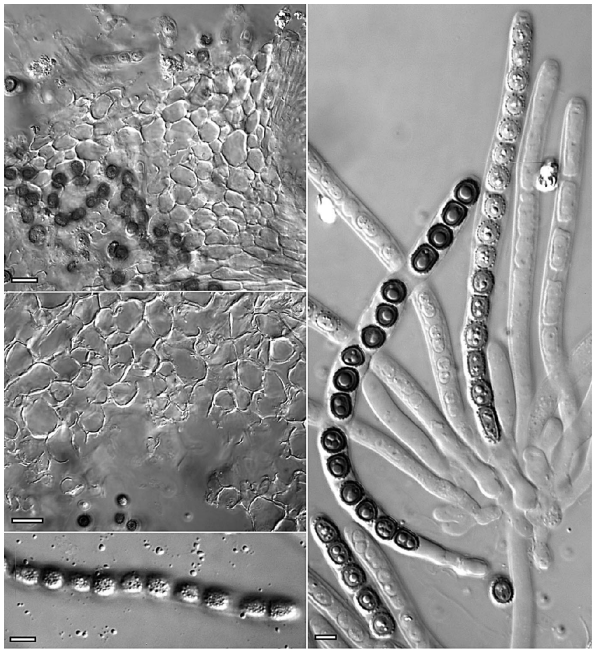
FIGS. 16–24. *Trichoderma crassum*. 16–18. Conidiophores. 19, 20, 23, 24. Gliocladium-like synanamorph. 21. Conidia. 22. Long conidiophores. 16–19, 21–23 = DAOM 164916 (ex-type), 20 = G.J.S. 98-179, 24 = G.J.S. 95-157. Scale bars: 16–20 = 10 μm, 21 = 5 μm, 22, 23 = 15 μm, 24 = 20 μm.

)6.0–6.5(–7.0) μm. Ascosporae bicellulares, verruculosae, verrucis magnitudine dissimilibus, ad septum disarticulatae, atrovirentes; parte distali globosa ad subglobosa, (4.2–)5.5–6.0(–6.7) × (4.5–)5.2–5.5(–7.0) μm, parte proximali cuneiformi ad cylindrica, (5.0–)5.7–6.2(–7.2) × (4.0–)4.7–5.2(–6.2) μm. Anamorphosis *Trichoderma* sp. Phialidis (7.2–)9.0–10.5(–15.2) × (2.7–)3.2–3.5(–4.5) μm; conidii viridia, glabra, (3.5–)4.0–4.5(–4.7) × (3.2–)3.5–3.7(–4.0) μm, ratio longitudo/crassitudo (1.0–)1.1–1.2(–1.4). Incrementum in agaro "PDA" dicto post 72 h 15 C = 14–19 mm, 20 C = 47–50 mm, 25 C = 63–72 mm, 30 C = 70–74 mm, 35 C = 4–5 mm. Holotypus BPI 1112894.

Anamorph.—*Trichoderma* sp.

Stromata scattered, pulvinate, nearly circular in outline, (0.8–)0.9–1.2(–1.5) mm diam (n = 10), ca 0.6 mm high (n = 10), broadly attached, surface smooth, with perithecial protuberances, light yellow, somewhat transparent, not changing color in KOH, ostiolar openings obvious due to the discharged

FIG. 25. *Hypocrea cremea* anamorph (holotype). Scale bars = 10 μm.



FIGS. 26–29. *Hypocrea cremea* teleomorph (holotype). 26. Tissue of surface of stroma. 27. Internal tissue below perithecia. 28. Ascospore ornamentation. 29. Asci and ascospores. Scale bars: 26 = 10 μm, 27 = 20 μm, 28, 29 = 5 μm.

green ascospores. Tissue of the stroma formed of hyaline angular cells that increase in size progressively from the surface to the internal tissue below perithecia. Stroma surface cells (5.2–)8.5–10.2(–14.7) μm diam (n = 30), walls 0.5–0.7(–1.0) μm thick (n = 25). Internal tissue below perithecia formed of cells (8.5–)13.7–17.2(–28.0) μm diam (n = 30), walls 0.7–1.0(–1.2) μm thick (n = 10). Perithecia completely immersed in stroma, generally closely aggregated, subglobose, 233–267 μm high, 138–198 μm wide (n = 5), wall composed of compacted cells, KOH−, 13–17(–19) μm thick (n = 10), ostiolar canal 50–65 μm long (n = 5). Asci cylindrical, (99–)104–110(–120) × (4.7–)6.0–6.5(–7.0) μm (n = 20), ascospores uniseriate. Part-ascospores green, warted, with warts of different sizes, dimorphic, distal part globose to subglobose, (4.2–)5.5–6.0(–6.7) × (4.5–)5.2–5.5(–7.0) μm, proximal part cuneiform to cylindrical, (5.0–)5.7–6.2(–7.2) × (4.0–)4.7–5.2(–6.2) μm (n = 30).

Colonies on CMD at 20 C after ca 1 wk flat, with discrete tufts 2–3 mm diam (n = 6) forming on the edges of the plate, no distinctive odor, no pigmentation of the agar. The branching pattern of the conidiophores irregular, branches not paired and generally longer toward the base, phialides cylindrical, broader in the middle, and constricted at the tip, formed in whorls of (1–)3(–5), (7.2–)9.0–10.5(–15.2) μm long, (2.7–)3.2–3.5(–4.5) μm at the widest point, (1.7–)2.0–2.5(–3.2) μm at the base, L/W (1.6–)2.7–3.2(–5.1) (n = 30), elongations of the conidiophore not common, fertile, terminating in 1 or 2 phialides (9.7–)10.5–12.7(–13.7) μm long, 2.7–3.2 μm at the widest point, (1.7–)2.2–2.5(–3.2) μm at the base, L/W (3.3–)3.5–4.2(–4.4) (n = 10); subtending hyphae cylindrical, (2.7–)3.2–3.7(–5.0) μm wide (n = 22). Conidia green, smooth, obovoid to subglobose, (3.5–)4.0–4.5(–4.7) × (3.2–)3.5–3.7(–4.0) μm, L/W (1.0–)1.1–1.2(–1.4) (n = 30). No chlamydospores observed.

Colonies on PDA at 25 C after ca 1 wk cottony, with no conidia formed until ca 2 wk, no pigmentation of agar and no distinctive odor. Colony radius after 3 d on PDA at 15 C: 14–19 mm, 20 C: 47–50 mm, 25 C: 63–72 mm, 30 C: 70–74 mm, and 35 C: 4–5 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 2–21 mm, 20 C: 27–48 mm, 25 C: 42–55 mm, 30 C: 55–63 mm, and 35 C: 2–7 mm (n = 3).

Habitat.—Decorticated wood.

Known distribution.—United States (New York).

Holotype.—UNITED STATES. NEW YORK: Hollyhock Hollow Sanctuary, Rarick Road, 3 miles South of Feura Bush, on decorticated wood, 21 Sep 1991, R. Lowen (BPI 1112894; cultures: G.J.S. 91-125 = ATCC MYA-2862 = CBS 111146 = DAOM 231312).

Additional specimen examined.—UNITED STATES. NEW YORK: Ulster County, Ashokan Camp, on decorticated wood, probably on a black pyrenomycete, 27 Sep 1998, P. Chaverri (culture: P.C. 14).

Notes.—This species is characterized by the semitransparent pale yellow stromata, ascospores with variable size warts, fast growth on PDA at 30 C and very slow

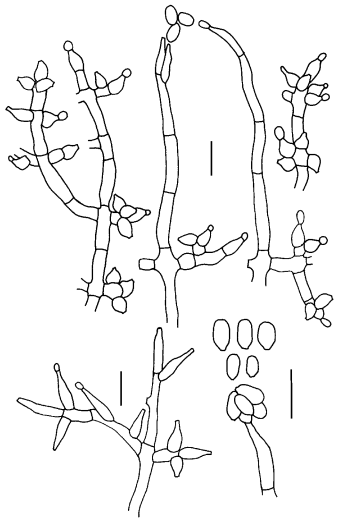
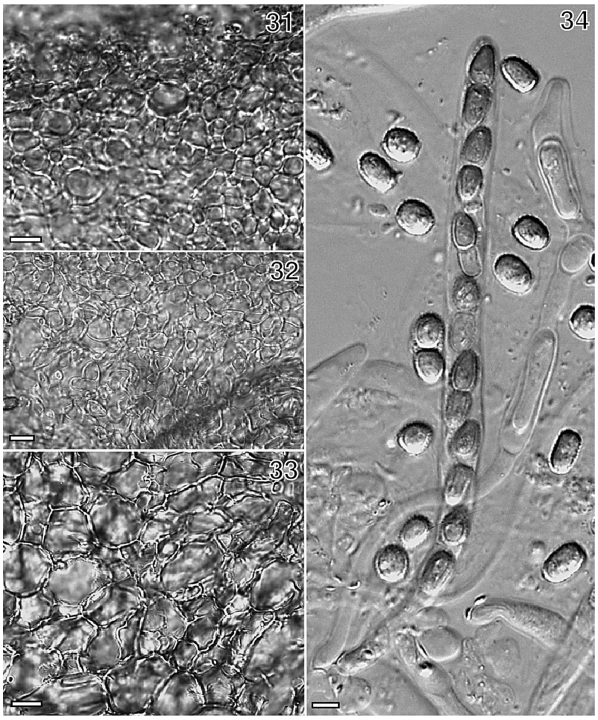


FIG. 30. *Hypocrea cuneispora* anamorph (holotype). Scale bars = 10 μm.



FIGS. 31–34. *Hypocrea cuneispora* teleomorph (holotype). 31. Tissue of surface of stroma. 32. Tissue below surface layer, between perithecia. 33. Internal tissue below perithecia. 34. Asci and ascospores. Scale bars: 31–33 = 10 μm, 34 = 5 μm.

or no growth at 35 C. *Hypocrea cremea* is a sister species to *H. surrotunda* based on RPB2 and EF-1α sequence data. Their anamorphs are almost indistinguishable: both have phialides that are attached to the subtending hyphae at wide angles, thus they both have morphology of sect. *Trichoderma*. *Hypocrea cremea* is distinguished from *H. surrotunda* by the faster growth rate and smaller conidia. In addition, the stroma of *H. cremea* is semitransparent and somewhat glossy whereas the stroma of *H. surrotunda* is opaque.

5. *Hypocrea cuneispora* Chaverri et Samuels, sp. nov. FIGS. 30–34, 142, 151, 171

Stromata albolutea, subsquarrosa, KOH+, (0.6–)0.7–1.3(–1.8) mm diam. Asci cylindrici, (109–)121–139(–149) × (5.2–)5.5–6.0(–6.5) μm. Ascosporae bicellulares, verruculosae, ad septum disarticulatae, atrovirentes; parte distali cuneiformi ad subglobosa, (5.5–)6.5–7.0(–8.0) × (4.2–)5.0–5.2(–6.0) μm, parte proximali cuneiformi, (6.0–)7.0–7.7(–10.0) × (3.7–)4.5–4.7(–5.5) μm. Anamorphosis *Trichoderma* sp. Phialidis (4.0–)6.0–7.2(–10.5) × (3.0–)3.7–4.0(–4.5) μm; conidii viridia, glabra, oblongata ad ellipsoidea, (4.5–)5.5–6.0(–7.2) × 2.5–)3.2–3.5(–4.0) μm, ratio longitudo/crassitudo (1.4–)1.6–1.8(–2.3). Holotypus BPI 1112864.

Anamorph.—Trichoderma sp.

Stromata aggregated or in pairs and adjacent stromata sometimes fusing, pulvinate, circular to irregular in outline, (0.6–)0.7–1.3(–1.8) mm diam (n = 10), (0.8–)0.9–1.0 mm high (n = 10), broadly attached, surface rough, with no perithecial protuberances, orange-brown to buff, becoming darker in KOH, ostiolar openings obvious due to the green ascospores. Outermost layer of stroma composed of dead cells, small, irregularly shaped, compact, that make the surface look rough or scaly. Layer below the outermost layer of stroma composed of angular cells, brownish, (4.7–)6.7–8.0(–12.5) μm diam (n = 30), walls (0.5–)0.7–1.0(–1.2) μm thick (n = 30). Tissue between perithecia and below the second layer composed of hyaline, angular cells, (6.0–)8.5–9.7(–12.2) μm diam (n = 30), walls 0.5–0.7(–1.0) μm thick (n = 30). Internal tissue below the perithecia formed of angular, hyaline cells (11.0–)17.2–20.7(–28.2) μm diam (n = 30), walls (0.5–)0.7–1.0 μm thick (n = 30). Perithecia completely immersed in stroma, generally closely aggregated or with some space in between, subglobose, (262–)271–305(–309) μm high, (156–)162–225(–250) μm wide (n = 10), wall composed of compacted cells, KOH−, (10–)14–18(–24) μm thick (n = 20), ostiolar canal (60–)63–83(–94) μm long (n = 10). Asci cylindrical, (109.0–)121–139(–149) × (5.2–)5.5–6.0(–6.5) μm (n = 10), ascospores uniseriate. Part-ascospores green, warted, generally dimorphic, distal part generally cuneiform or subglobose, sometimes barrel-shaped (5.5–)6.5–7.0(–8.0) × (4.2–)5.0–5.2(–6.0) μm, proximal part generally cuneiform, sometimes barrel-shaped or ellipsoidal, (6.0–)7.0–7.7(–10.0) × (3.7–)4.5–4.7(–5.5) μm (n = 30).

Colonies on CMD at 20 C after ca 1 wk flat, with discrete small tufts <1 mm diam that form on the edges of the plate, conidia produced after ca 2 wk, no

distinctive odor, no pigmentation of the agar. Conidiophores generally producing short branches that generally do not rebranch, with some phialides arising directly from the conidiophore. Phialides short, ampulliform, formed in whorls of (1–)2–3(–5), (4.0–)6.0–7.2(–10.5) μm long, (3.0–)3.7–4.0(–4.5) μm at the widest point, (1.5–)2.5–2.7(–3.7) μm at the base, L/W (1.0–)1.6–2.0(–3.4) (n = 30), apical elongations of the conidiophore common, sterile or fertile, with 1 or 2 cylindrical phialides, slightly broader at the middle, constricted at the tip, 11–15 μm long, 2.2–3.2 μm at the widest point, 1.7–2.7 μm at the base, L/W 3.3–5.1 (n = 10); subtending hyphae cylindrical, (2.7–)3.5–4.5(–4.7) μm wide (n = 10); a verticilliumlike synanamorph sometimes observed. Conidia green, smooth, oblong to ellipsoidal, (4.5–)5.5–6.0(–7.2) × (2.5–)3.2–3.5(–4.0) μm, L/W (1.4–)1.6–1.8(–2.3) (n = 30). Abundant chlamydospores formed after 3 wk, intercalary or terminal, globose to subglobose, (8.7–)10.2–11.7(–13.2) μm diam (n = 15).

Colonies on PDA at 25 C after ca 1 wk flat, with scant aerial mycelium, conidia not formed until ca 2 wk after inoculation, no pigmentation of the agar and no distinctive odor. Colony radius after 3 d on PDA at 15 C: 14–18 mm, 20 C: 32–35 mm, 25 C: 41–43 mm, 30 C: 29–32 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 9–12 mm, 20 C: 31–34 mm, 25 C: 42–45 mm, 30 C: 19–23 mm, and 35 C: 0 mm (n = 3).

Habitat.—Decorticated wood.

Known distribution.—United States (Louisiana and Virginia).

Holotype.—UNITED STATES. VIRGINIA: Giles County, Cascades Recreation site, 4 miles North of Pembroke, Little Stony Creek, 37° 2′ N, 80° 35′ W, alt. 840 m, on decorticated wood, 18 Sep 1991, G.J.S., C.T.R., S. Huhndorf, S. Rehner, M. Williams (BPI 1112864; cultures: G.J.S. 91-93 = ATCC MYA-2863 = CBS 111148 = DAOM 231313).

Additional specimen examined.—UNITED STATES. LOUISIANA: East Baton Rouge Parish, Burden Plantation, on hymenium of *Phellinus* sp., 30 Jul 1991, M. Blackwell, D. Hawksworth, G.J.S. (BPI 1112810, culture: G.J.S. 91-35).

Notes.—This species is characterized by large ascospores and large ellipsoidal conidia. The two North American specimens examined sometimes produced stromata on CMD. *Hypocrea cuneispora* resembles *H. aureoviridis* f. *macrospora*, which was described from Japan, in the large ascospores and large ellipsoidal conidia. These two species differ in that *H. a.* f. *macrospora* has slightly longer and more slender phialides and slightly smaller ascospores (Doi 1972). Unfortunately, the ex-type culture of *H. a.* f. *macrospora* is not available. *Hypocrea cuneispora* is a sister species of *T. longipile* based on RPB2 and EF-1α sequence data. Both species have ellipsoidal conidia with a length/width ratio 1.6–2.0 and the conidiophore branching pattern and phialide distribution are very similar. *Hypocrea cuneispora* anamorph can be distinguished from *T. longipile* by the longer phialides and the faster growth on PDA and SNA.

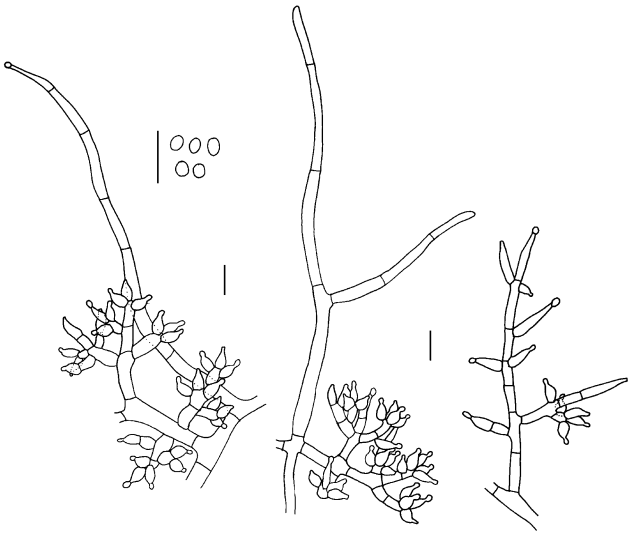


FIG. 35. *Hypocrea estonica* anamorph (holotype). Scale bars = 10 μm.

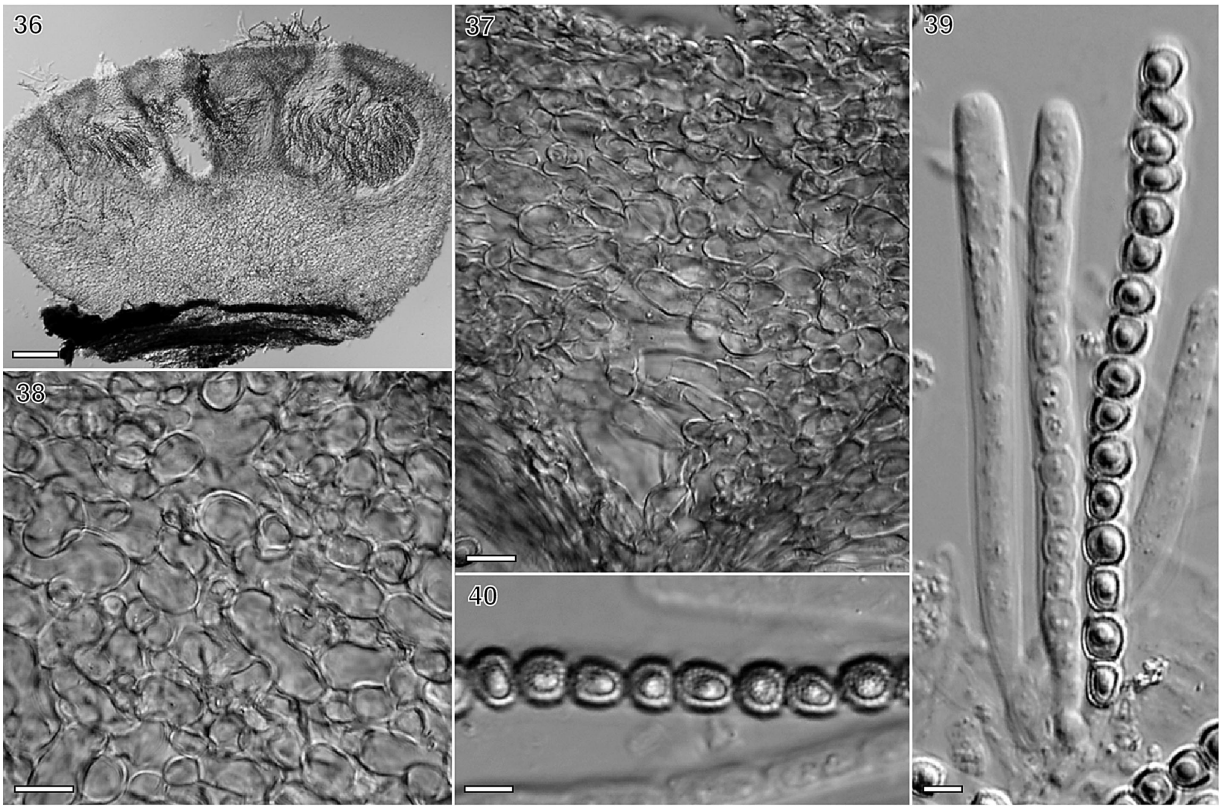
6. *Hypocrea estonica* Chaverri et Samuels, sp. nov.

FIGS. 35–40, 143, 152, 172

Stromata albolutea, (0.4–)0.6–0.8(–1.0) mm diam. Ascosporae bicellulares, verruculosae, ad septum disarticulatae, atrovirentes; parte distali subglobosa ad oblonga, (3.7–)5.0–5.2(–6.0) × (3.5–)5.0–5.2(–5.7) μm, parte proximali cuneiforme, (4.2–)5.0–5.2(–6.2) × (3.2–)4.2–4.5(–5.2) μm. Anamorphosis *Trichoderma* sp. Phialidis (4.5–)6.5–7.0(–11.0) × (3.0–)3.5–3.7(–4.5) μm; conidii viridia, glabra, (3.5–)4.0–4.2(–5.0) × (2.5–)3.2–3.5(–4.2) μm, ratio longitudo/crassitudo (1.0–)1.2–1.3(–1.7). Incrementum in agaro "PDA" dicto post 72 h 15 C = 5–16 mm, 20 C = 15–23 mm, 25 C = 25–40 mm, 30 C = 4–13 mm, 35 C = 0 mm. Holotypus BPI 744577.

Anamorph.—*Trichoderma* sp.

Stromata scattered, solitary, pulvinate, circular to irregular in outline, (0.4–)0.6–0.8(–1.0) mm diam (n = 20), (0.2–)0.4–0.5(–0.6) mm high (n = 20), broadly attached, surface smooth, perithecial protuberances visible, pale yellow, becoming brownish in KOH, ostiolar openings obvious due to the green ascospores. Outermost layer of stroma composed of compacted angular cells, (3.5–)5.2–6.0(–8.5) μm diam (n = 30), walls 0.5–0.7(–1.2) μm thick (n = 20). Tissue between perithecia and below the outermost layer composed of hyaline cells, of *textura angularis* to *epidermoidea,* (4.2–)6.0–7.5(–11.0) μm diam (n = 30),



FIGS. 36–40. *Hypocrea estonica* teleomorph. 36. Section of stroma. 37. Tissue of surface of stroma. 38. Internal tissue below perithecia. 39, 40. Asci and ascospores. 36–38 = G.J.S. 96-129 (holotype), 39, 40 = G.J.S. 96-3. Scale bars: 36 = 150 μm, 37, 38 = 10 μm, 39, 40 = 5 μm.

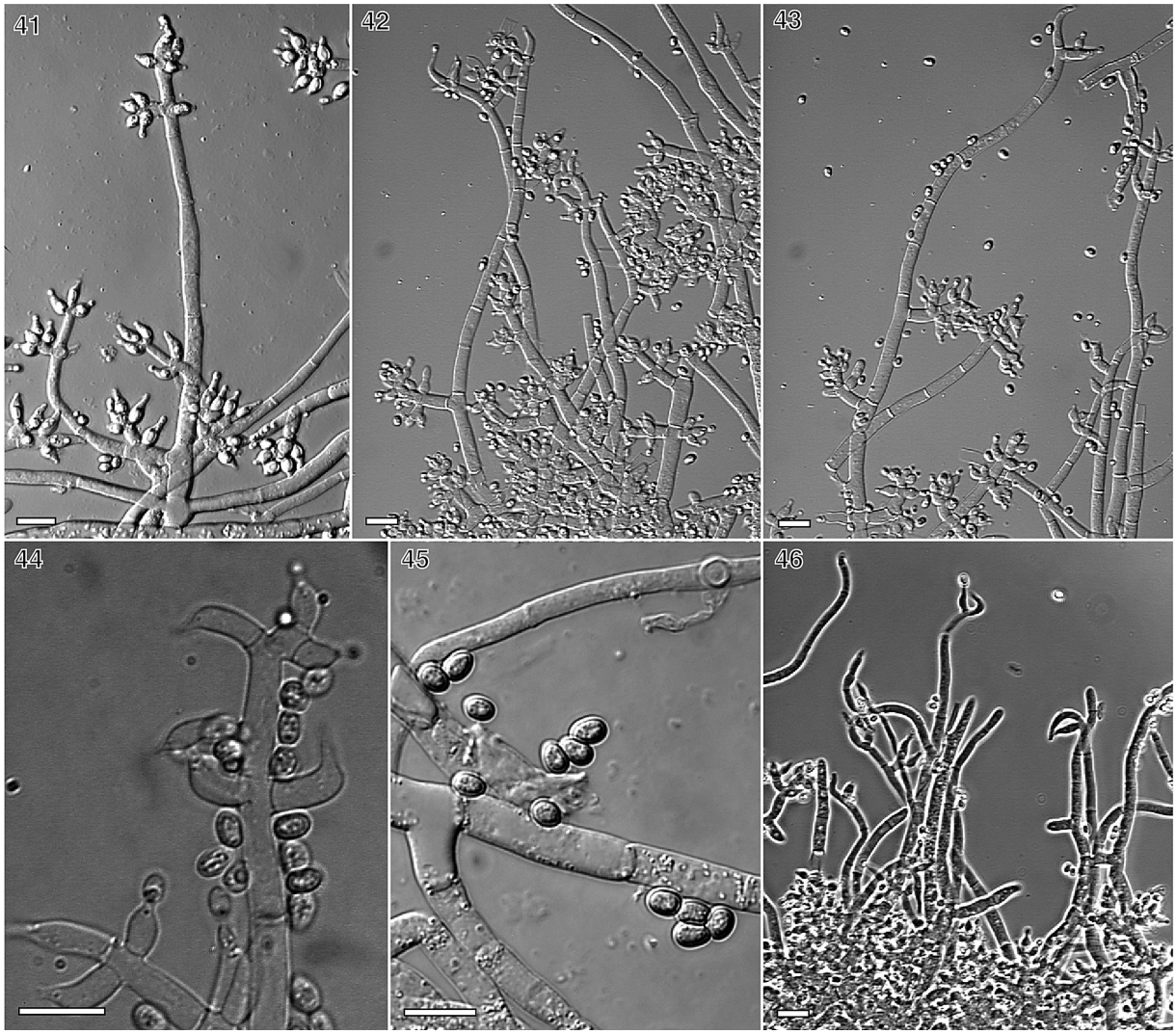
walls 0.5(–0.9) μm thick (n = 20). Internal tissue below the perithecia of *textura angularis* to *epidermoidea* (5.0–)7.5–9.0(–13.2) μm diam (n = 40), walls 0.5–0.7(–1.0) μm thick (n = 30). Perithecia immersed in the stroma, generally closely aggregated or slightly separated, subglobose, (159–)222–281(–348) × (116–)148–199(–237) μm (n = 15), wall composed of compacted cells, turning brownish in KOH, (11–)15–18(–22) μm thick (n = 20), ostiolar canal (46–)54–79(–89) μm long (n = 10). Asci cylindrical, uniseriate, (85–)91–96(–103) × (5.0–)5.7–6.2(–7.2) μm (n = 20). Part-ascospores green, warted, dimorphic, distal part subglobose to oblong (3.7–)5.0–5.2(–6.0) × (3.5–)5.0–5.2(–5.7) μm, proximal part cuneiform, (4.2–)5.0–5.2(–6.2) × (3.2–)4.2–4.5(–5.2) μm (n = 40).

Colonies on CMD at 20 C after ca 1 wk flat, with discrete, pulvinate compact tufts 1–2 mm diam (n = 10) forming at the edges of the plate, each tuft having many long hairs projecting, conidia produced after ca 2 wk, no distinctive odor, no pigmentation of the agar. Phialides short, ampulliform, formed in whorls of (1–)3–4(–5), (4.5–)6.5–7.0(–11.0) μm long, (3.0–)3.5–3.7(–4.5) μm at the widest point, (1.7–)2.2–2.5(–4.2) μm at the base, L/W (1.2–)1.7–1.9(–3.6) (n = 60), apical elongations of the conidiophore common, fertile or sterile, with one terminal phialide, slightly broader at the middle, constricted at the tip, (8.0–)14.7–18.2(–25.0) μm long, (2.0–)2.5–3.0(–3.7) μm at the widest point, (2.0–)2.5–2.7(–3.5) μm at the base, L/W (5.3–)5.4–7.9(–8.0) (n = 30); subtending hyphae cylindrical, (2.7–)3.5–4.0(–5.5) μm wide (n = 40); a verticillium-like synanamorph sometimes observed. Conidia green, smooth, ellipsoidal, (3.5–)4.0–4.2(–5.0) × (2.5–)3.2–3.5(–4.2) μm, L/W (1.0–)1.2–1.3(–1.7) (n = 50). No chlamydospores observed.

Colonies on PDA at 25 C after ca 1 wk flat, with scant aerial mycelium, conidia forming slowly, only after ca 2 wk, no pigmentation of the agar, no distinctive odor. Colony radius after 3 d on PDA at 15 C: 5–16 mm, 20 C: 15–23 mm, 25 C: 25–40 mm, 30 C: 4–13 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 2–12 mm, 20 C: 4–17 mm, 25 C: 9–25 mm, 30 C: 2–5 mm, and 35 C: 0 mm (n = 3).

Habitat.—Fungicolous and on blackened decorticated wood, probably growing on another fungus.

Known distribution.—Estonia.



FIGS. 41–46. *Trichoderma fertile*. 41–43, 46. Conidiophores and elongations. 44. Phialides and conidia. 45. Conidia. 41–45 = Isolate DAOM 167161 (ex-type), 46 = DAOM 167070. Scale bars: 41–46 = 10 μm.

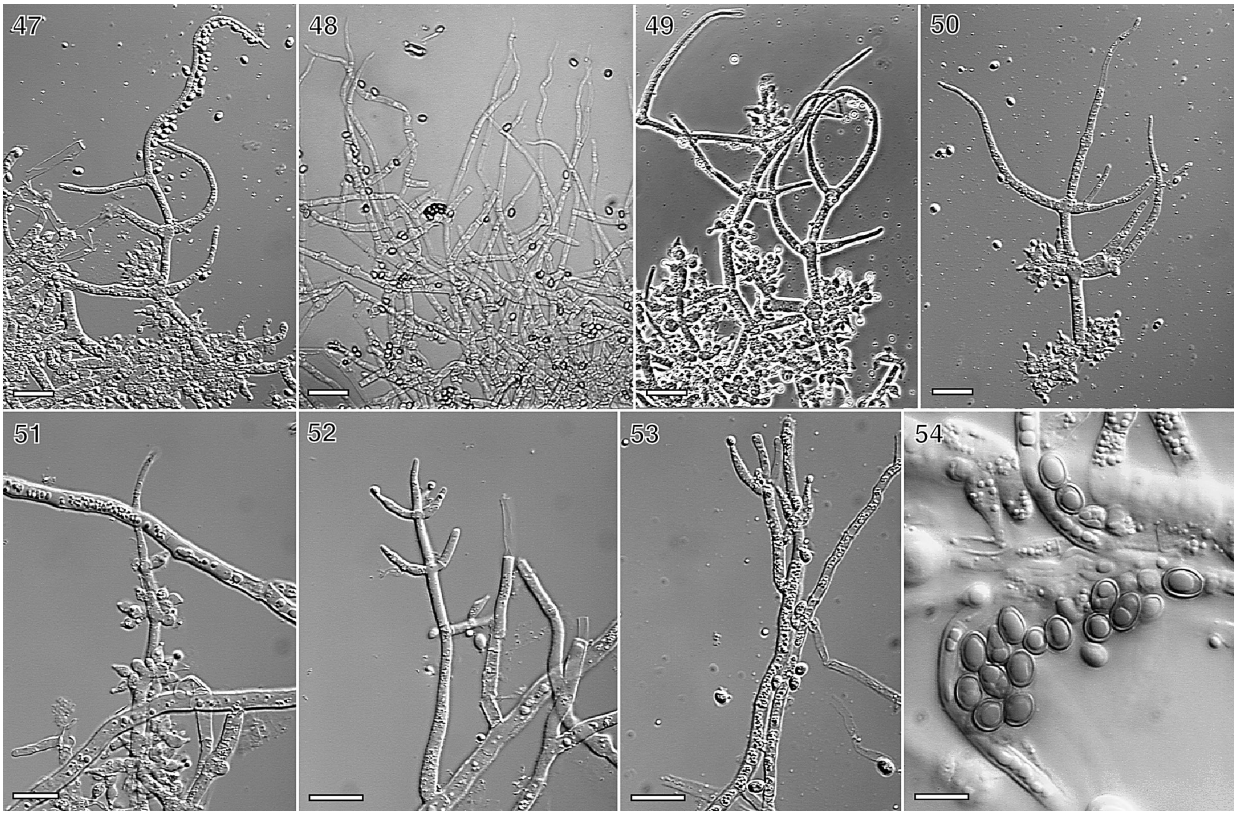
Holotype.—ESTONIA. VALGAMAA COMMUNITY: Vaabina, on hymenium of *Hymenochaete tabacina*, 20 Apr 1996, K. Põldmaa (BPI 744577; cultures: G.J.S. 96-129 = ATCC MYA-2864 = CBS 111147 = DAOM 231314; ISOTYPE: TAA 161844).

Additional specimen examined.—ESTONIA. JÃRVAMAA DISTRICT: Tũri Forestry, Lepametsa, on blackened decorticated wood of *Betula* sp., 21 Sep 1995, V. Kastanje (TAA 161780 = BPI 744462, culture: G.J.S. 96-3).

Notes.—This species is characterized by slow growth, especially at 30 C. Most species of *Hypocrea/Trichoderma* grow well at 30 C. *Hypocrea estonica* phylogenetically is closely related to *H. ceramica* based on RPB2 and EF-1α molecular sequence data.

7. *Trichoderma fertile* Bissett, Can. J. Bot. 69: 2382. 1991. FIGS. 41–46, 153, 173

Colonies on CMD at 20 C after ca 2 wk flat, with conidia forming abundantly around the margin of the colony in pustules and in the aerial mycelium. Pustules ill-defined, cottony, less than 1 mm diam, with numerous terminally fertile conidiophores protruding. Conidiophores typically consisting of a fertile extension with slender phialides arising at the tip; fertile secondary branches arising from a lower position on the conidiophore, short, paired or solitary, with one or a few phialides terminating each branch and phialides arising directly from the branch, or the secondary branch rebranching to produce typically paired tertiary branches, each of which terminating in a whorl of a few phialides. Fertile extensions of conidiophores straight, smooth, thin-walled, producing one or a few slender phialides at the tip with a long internode between the tip and the next lower branch. Basal phialides tending to be held in more or less divergent



FIGS. 47–54. *Trichoderma hamatum* (DAOM 167057, ex-type). 47–51. Conidiophores and elongations. 52, 53. Verticillium-like synanamorph. 54. Conidia. Scale bars: 47–50 = 20 μm, 51–53 = 15 μm, 54 = 10 μm.

whorls, conspicuously swollen in the middle or nearly doliiform, (4.5–)5.2–6.5(–7.5) μm long, (2.7–)3.5–4.7 μm at the widest point, (1.5–)2.0–2.7(–3.5) μm wide at the base, L/W (1.2–)1.3–2.0(–2.1), arising from a cell 3–6 μm wide (n = 30). Terminal phialides tending to be straight or slightly hooked, cylindrical or slightly enlarged in the middle, (6.5–)8.5–9.5(–12.0) μm long, 2.7–4.0 μm at the widest point, (1.5–)2.0–2.2(–3.0) μm wide at the base (n = 15). Conidia green, smooth, ellipsoidal, (2.5–)3.5–4.0(–4.7) × (2.2–)2.5–3.0(–3.2) μm, L/W (1.1–)1.4–1.5(–1.9) (n = 30). Chlamydospores not observed.

Colonies on PDA at 25 C after ca 2 wk slightly cottony, with conidia formed densely in a central disk and concentric rings of conidial production beginning to develop; no pigmentation of the agar, no distinctive odor. Colony radius after 3 d on PDA at 15 C: 8–20 mm, 20 C: 19–40 mm, 25 C: 25–50 mm, 30 C: 18–55 mm, and 35 C: 0 mm (n = 9). Colony radius after 3 d on SNA at 15 C: 0–10 mm, 20 C: 13–23 mm, 25 C: 21–31 mm, 30 C: 13–40 mm, and 35 C: 0–1 mm (n = 9).

Habitat.—Soil and herbaceous tissue.

Known distribution.—Canada and U.K.

Cultures examined.—CANADA. ALBERTA: Forestburg, from wheat soil, Nov 1976, S. Visser (DAOM 167161, ex-type). QUEBEC: Lacolle, from pine forest soil, Jul 1975, P. Widden (DAOM 167070).

Notes.—*Trichoderma fertile* morphologically is similar to *T. spirale*. *Trichoderma fertile* can be distinguished from *T. spirale* by its straight conidiophore elongation that is generally unbranched, and when branched then with a short fertile branch near the tip. *Trichoderma fertile* has slightly smaller conidia and slightly longer phialides. *Trichoderma fertile* is closely related to *T. oblongisporum* and *H. semiorbis* based on RPB2 and EF-1α sequence data.

8. *Trichoderma hamatum* (Bonord.) Bain., Bull. Soc. Mycol. Fr. 22: 131. 1906. FIGS. 47–51, 154, 174

≡ *Verticillium hamatum* Bonord., Handb. allg. Mykol. p. 97. 1851.

≡ *Pachybasium hamatum* (Bonord.) Sacc., Rev. Mycol. 7: 160. 1885

≡ *Phymatotrichum hamatum* (Bonord.) Oudem., Ned. Kruidk. Archf. 3(2): 908. 1903.

Colonies on CMD at 20 C after ca 1 wk with abundant pustules, scattered, pulvinate, 0.5–1.5(–3.0) mm diam (n = 20), and with a synanamorph forming in the scantily produced aerial mycelium. Conidiophores in pustules comprising a sterile elongation with the

fertile part arising near the base. Phialides arising from short lateral branches at the base of the elongation. Lateral branches typically comprising one or a few broad cells with phialides arising at the tip and along the length, or secondary branches sometimes arising from lateral branches also as single cells from which phialides arise. The internode between branches often short and the ovoid phialides densely clustered, then the fertile part of the conidiophore giving the appearance of a cluster of grapes. Main axis and branches conspicuously broad, (2.5–)3.2–4.5(–5.0) μm wide (n = 30). Conidiophore apical elongations sterile, conspicuously extending beyond the surface of the pustules, sterile secondary branches arising below the tip or unbranched, straight, smooth, thin-walled. Phialides ampulliform, (4.5–)5.2–7.2(–9.5) μm long, (2.7–)3.2–4.2(–5.5) μm wide at the widest point, (1.5–)2.0–3.0(–3.7) μm at the base, L/W (1.2–)1.3–2.1(–3.1), densely clustered on broad subtending hyphae (2.5–)3.2–4.5(–5.0) μm wide (n = 30). Conidia smooth, green, ellipsoidal, (3.9–)4.2–5.0(–5.8) × (2.4–)2.7–3.0(–3.4), L/W (1.2–)1.5–1.6(–2.1) (n = 30). Synanamorph forming sparingly in the aerial mycelium, conidiophores ca 60 μm long, ca 5 μm wide at the base, smooth, phialides arising in 1–2 verticils below the tip. Phialides tapering slightly from base to tip or somewhat enlarged in the middle, (5.7–)6.7–13.2(–18.5) μm long, (2.0–)2.5–3.5 μm at the widest point, 2.0–3.0(–3.2) μm at the base, arising from a cell 2.5–3.0(–3.5) μm wide (n = 30). Conidia ellipsoidal, 4.0–5.0(–5.5) × (2.7–)3.0–3.5(–4.0) μm, smooth, held in drops of clear, watery green liquid. Chlamydospores sometimes formed, terminal and intercalary, subglobose to globose, (7–)10–13(–16) μm diam (n = 20).

Colonies on PDA at 25 C after ca 1 wk with dense mycelium, conidia forming in concentric rings, no diffusing pigment or distinctive odor produced. Colony radius after 3 d on PDA at 15 C: 8–14 mm, 20 C: 25–34 mm, 25 C: 45–52 mm, 30 C: 50–57 mm, and 35 C: 0–6 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 3–10 mm, 20 C: 22–25 mm, 25 C: 30–47 mm, 30 C: 36–55 mm, and 35 C: 0–5 mm (n = 3).

Habitat.—Soil, wood and herbaceous tissue.

Known distribution.—Probably cosmopolitan.

Cultures examined.—CANADA. ONTARIO: Cochrane, from soil, May 1984, M. T. Dumas (DAOM 199079). QUEBEC: Lacolle, from spruce forest soil, Apr–Jun 1977, P. Widden (DAOM 167057, ex-neotype).

Notes.—*Trichoderma hamatum* is morphologically almost identical to *T. pubescens*. They both have sterile and branched conidiophore elongations and indistinguishable conidiophore branching and phialide distribution. *Trichoderma hamatum* can be distinguished from *T. pubescens* by the slightly larger conidia, slightly longer phialides and in their respective growth rates, where *T. hamatum* grows significantly faster than *T. pubescens*, and the optimum growth temperature on SNA is 30 C and 25 C, respectively (FIG. 1). We do not discard the possibility that *T. hamatum* and *T. pubescens* are synonyms. Bissett (1991b) distinguished *T. hamatum* from the morphologically similar *T. strigosum* and *T. pubescens* by nonfertile conidiophore elongations, which are stout, coiled and undulate or hamate. In addition, RPB2 and EF-1α gene genealogies place *T. hamatum* as a sister species to *T. pubescens*, and closely related to *T. strigosum* and *H. rufa/T. viride* Pers.

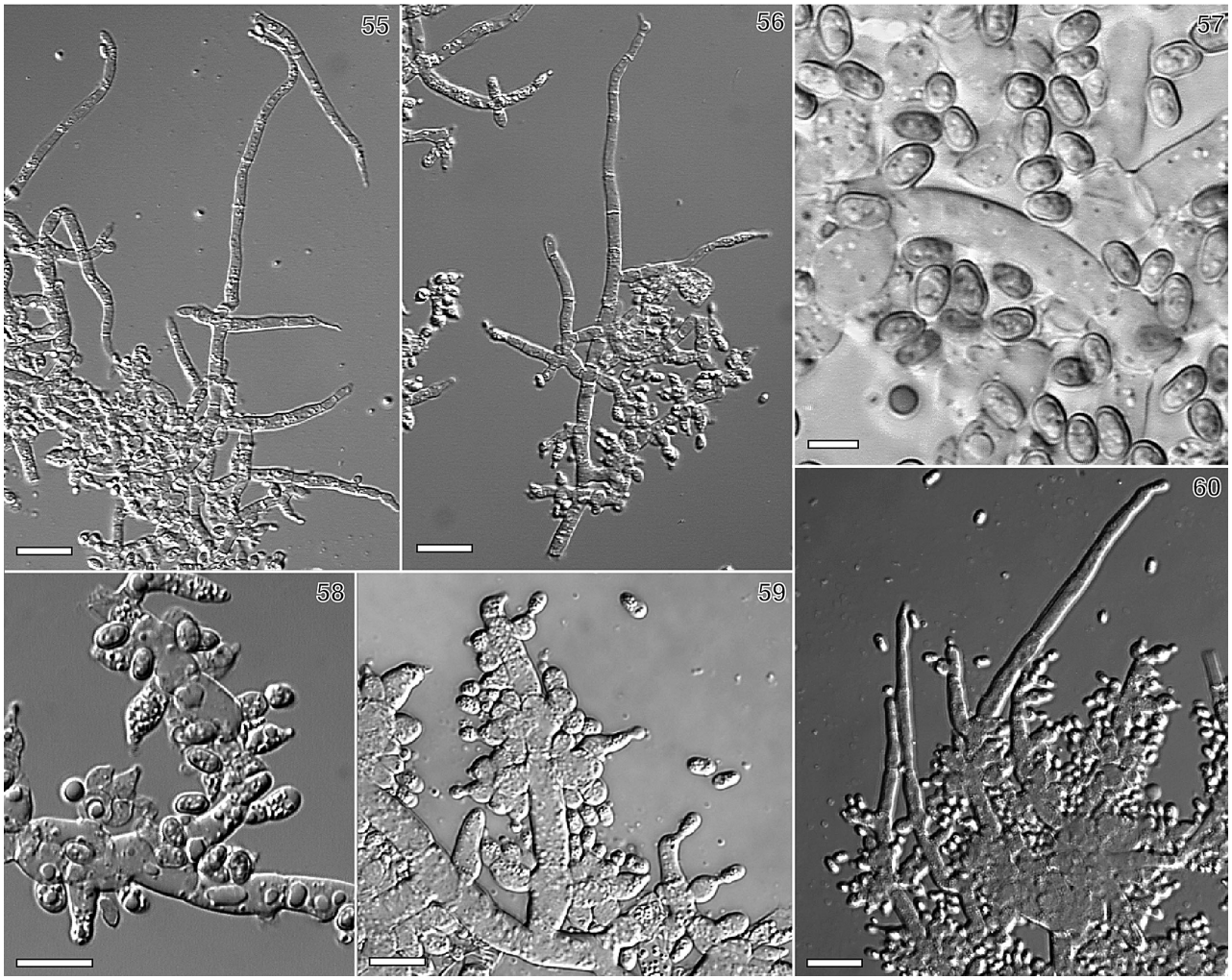
9. *Trichoderma longipile* Bissett, Can. J. Bot. 69: 2395. 1991.

Notes.—The culture that we examined remained sterile, therefore only growth data are presented. A complete description and illustrations are provided by Bissett (1991b). Colony radius after 3 d on PDA at 15 C: 8 mm, 20 C: 18–22 mm, 25 C: 27–30 mm, 30 C: 17–23 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 5–12 mm, 20 C: 18–22 mm, 25 C: 23–28 mm, 30 C: 23–25 mm, and 35 C: 0 mm (n = 3). *Trichoderma longipile* resembles *T. oblongisporum*, in conidial and phialide size, conidiophore branching and phialide distribution. Even though these two species are not phylogenetically closely related, based on RPB2 and EF 1-α molecular data, the morphology is almost indistinguishable. Bissett (1991b) distinguished *T. longipile* from *T. oblongisporum* by its branched and flexuous conidiophore elongations.

Culture examined.—CANADA. QUEBEC: Cantley, on *Ulmus* sp. log, 21 Sep 1980, H.L. Dickson, N. Grainger (DAOM 177227-1a, ex-type).

10. *Trichoderma oblongisporum* Bissett, Can. J. Bot. 69: 2398. 1991. FIGS. 55–60, 155, 175

Colonies on CMD at 20 C after ca 1 wk with pustules forming in a broad band around the margin of the Petri dish, hemispherical, easily removed from the agar, aggregated, 0.5–1.0(–1.5) mm diam, hairs and protruding conidiophores arising from all over the pustule. Conidiophores in pustules, branching irregular, usually wider at the base, branches usually attached at wide angles, with a long fertile or sterile conidiophore elongation. Conidiophore elongations straight, generally branching once or twice immediately above the fertile part of the conidiophore, bearing a single terminal drop of conidia. Phialides arising from subtending cells or directly from the conidiophore branch, ampulliform, (4.7–)5.5–6.0(–8.0) μm long, (3.0–)3.5–4.0(–4.7) μm wide at the widest point,



FIGS. 55–60. *Trichoderma oblongisporum* (DAOM 167085). 55, 56, 59, 60. Conidiophores and elongations. 57. Conidia. 58. Phialides and conidia. Scale bars: 55, 56, 60 = 15 μm, 57 = 5 μm, 58, 59 = 10 μm.

(1.7–)2.5–3.0(–4.5) μm at the base, L/W (1.0–)1.4–1.7(–2.5) (n = 30); subtending hyphae (2.7–)3.5–4.0(–5.0) μm wide (n = 30). Conidia smooth, green, ellipsoidal to oblong, (3.5–)4.5–4.7(–5.2) × (2.5–)2.7–3.0(–3.5), L/W (1.3–)1.6(–1.9) (n = 30). Chlamydospores not observed.

Colonies on PDA at 25 C after ca 1 wk with conidia forming in the middle of the colony and in a ring, no diffusing pigment or distinctive odor produced. Colony radius after 3 d on PDA at 15 C: 13–16 mm, 20 C: 23–29 mm, 25 C: 26–28 mm, 30 C: 20–25 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 3–4 mm, 20 C: 10–14 mm, 25 C: 20–21 mm, 30 C: 11–15 mm, and 35 C: 0 mm (n = 3).

Habitat.—Soil and wood.

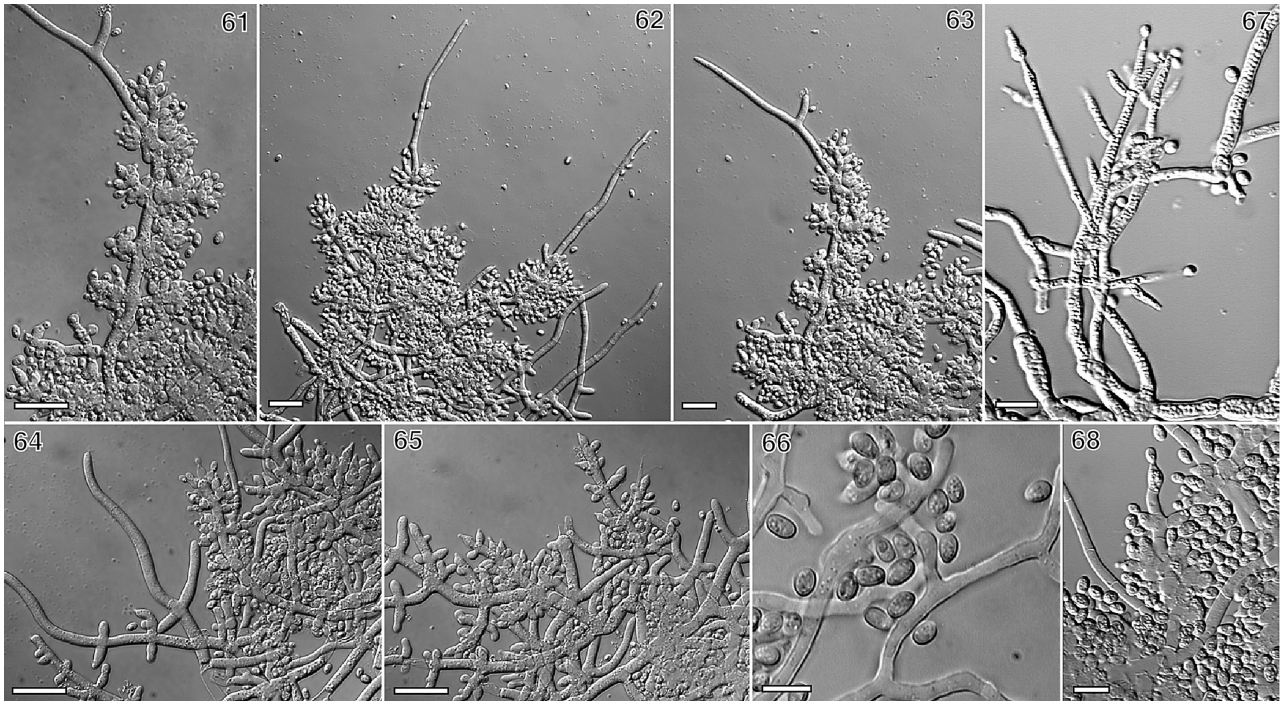
Known distribution.—Canada.

Cultures examined.—CANADA. ALBERTA: Heart Mountain, near Canmore, from soil, 6 Jul 1968, J. Bissett (J.B. 58) (DAOM 167085). BRITISH COLUMBIA: Pacific Rim National Park, on *Thuja plicata* wood, 19 Apr 1980, N. Grainger (DAOM 176226, ex-type).

Notes.—*Trichoderma oblongisporum* can be distinguished from *T. longipile* in having somewhat straight, generally unbranched conidiophore elongations. This species is closely related to *H. semiorbis* and *T. fertile* based on RPB2 and EF-1α gene phylogenies.

11. *Trichoderma pubescens* Bissett, Can. J. Bot. 69: 2405. 1991. FIGS. 61–68, 156, 176

Colonies on CMD at 20 C after ca 1 wk forming pustules sparsely around the margin of the colony, pustules 0.5–1 mm diam, having a ''spiky'' aspect because of the sterile conidiophore apical elongations, pustules easily removed from the agar; conidiophore elongations sterile, branched, arising from all over the pustule. Phialides ampulliform, short and wide, crowded on short lateral branches at the base of sterile elongations of the conidiophores, (4.1–)5.5–6.0(–



FIGS. 61–68. *Trichoderma pubescens* (DAOM 166162, ex-type). 61–65. Conidiophores and elongations. 66. Conidia. 67. Verticillium-like synanamorph. 68. Phialides and conidia. Scale bars: 61–65 = 20 μm, 66–68 = 10 μm.

7.7) μm long, (2.5–)3.5–3.7(–4.2) μm at the widest point, (1.7–)2.2–2.5(–3.5) μm at the base, L/W (1.2–)1.5–1.7(–2.4); subtending hyphae (2.7–)3.7–4.0(–5.5) μm wide (n = 30). Conidia green, smooth, ellipsoidal, (3.5–)4.0–4.2(–5.0) × (2.2–)2.7–3.0(–3.2) μm, L/W (1.3–)1.5–1.6(–1.9) (n = 30). Synanamorph abundant in the aerial mycelium apart from the pustules, conidia held in drops of clear green liquid; phialides held in poorly developed verticils. Phialides of synanamorph nearly cylindrical or tapering slightly from base to tip (8.2–)11.0–13.0(–16.5) μm long, (2.0–)2.5–2.7(–3.5) μm at the widest point, (1.5–)2.0–2.5(–3.7) μm at the base, L/W (2.4–)4.2–5.0(–6.8) (n = 25); conidia (3.7–)4.2–4.5(–6.5) × (2.2–)2.7–3.0(–3.2) μm, L/W (1.2–)1.5–1.6(–2.2) (n = 30). Chlamydospores abundant within 1 wk, almost entirely intercalary within hyphal cells, subglobose to clavate, (5.5–)7.0–8.2(–13.2) × (6.0–)7.2–8.0(–11.0) μm (n = 30).

Colonies on PDA at 25 C after ca 1 wk with conidia forming in dark concentric rings, no pigment and no distinctive odor detected. Colony radius after 3 d on PDA at 15 C: 8–9 mm, 20 C: 21–29 mm, 25 C: 32–33 mm, 30 C: 36–40 mm, and 35 C: 0–1 (n = 3). Colony radius after 3 d on SNA at 15 C: 2–7 mm, 20 C: 15–17 mm, 25 C: 25–27 mm, 30 C: 20–22 mm, and 35 C: 0–2 mm (n = 3).

Habitat.—Soil.

Known distribution.—United States (North Carolina); known only from the type locality.

Culture examined.—UNITED STATES. NORTH CAROLINA: Raleigh, from hardwood forest soil, Apr 1969, R. M. Danielson (DAOM 166162, ex-type).

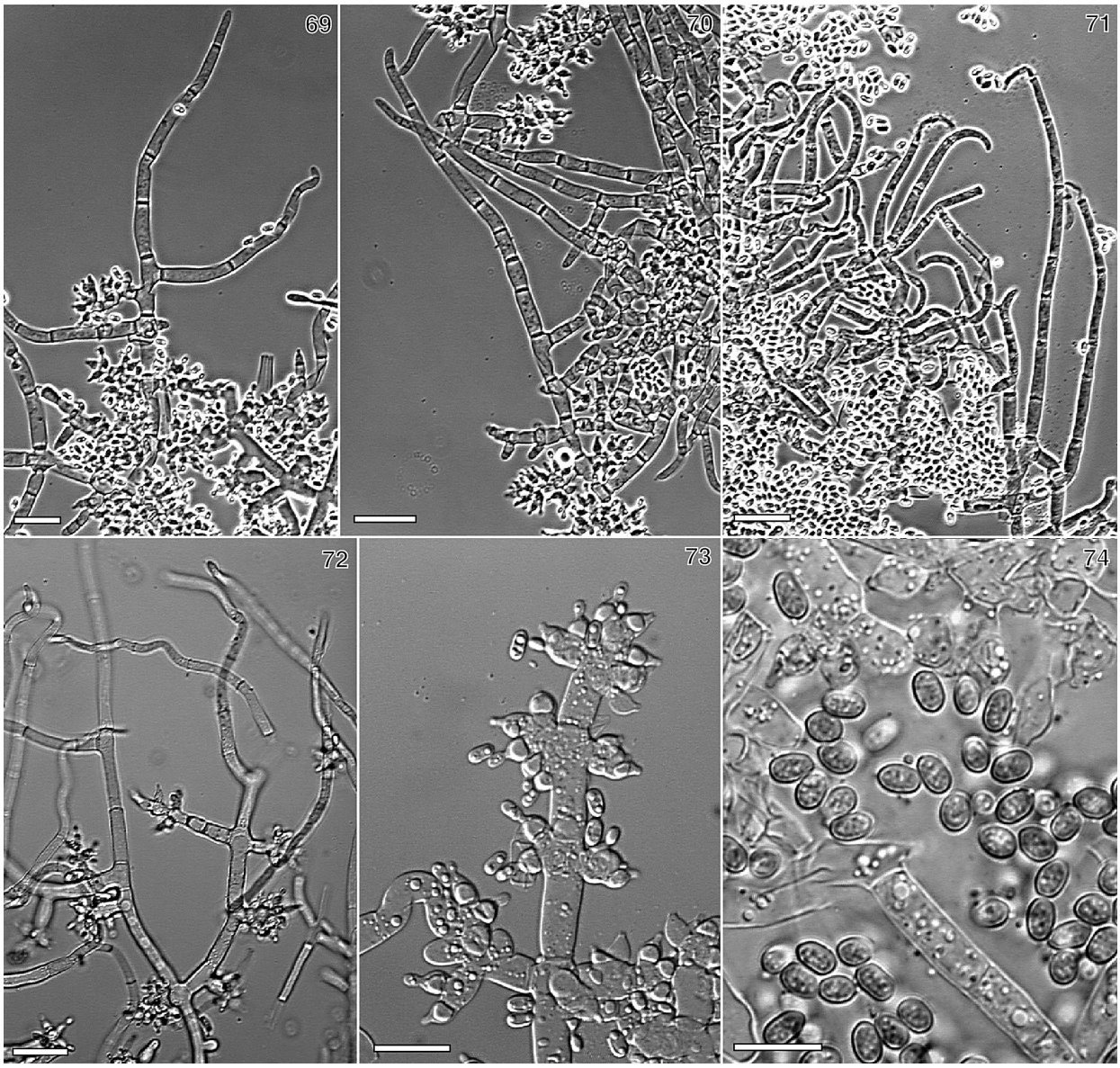
Notes.—*Trichoderma pubescens* is almost indistinguishable from *T. hamatum*. The main difference between these two species is in their conidiophore elongations. *Trichoderma pubescens* has thin, flexuous, branched conidiophore elongations. In addition, *T. pubescens* has slightly smaller phialides and conidia and slower growth on PDA at 30 C. RPB2 and EF-1α phylogenies place *T. pubescens* as a sister species to *T. hamatum* and closely related to *T. strigosum* and *H. rufa*.

12. *Hypocrea semiorbis* (Berk.) Berk. in Hooker, The botany of the Antarctic voyage. III. Flora Tasmaniae. 2: 278. 1859. FIGS. 69–81, 144, 157, 177

≡ *Sphaeria semiorbis* Berkeley, London J. Bot. 2: 146. 1840.

Anamorph.—*Trichoderma* sp.

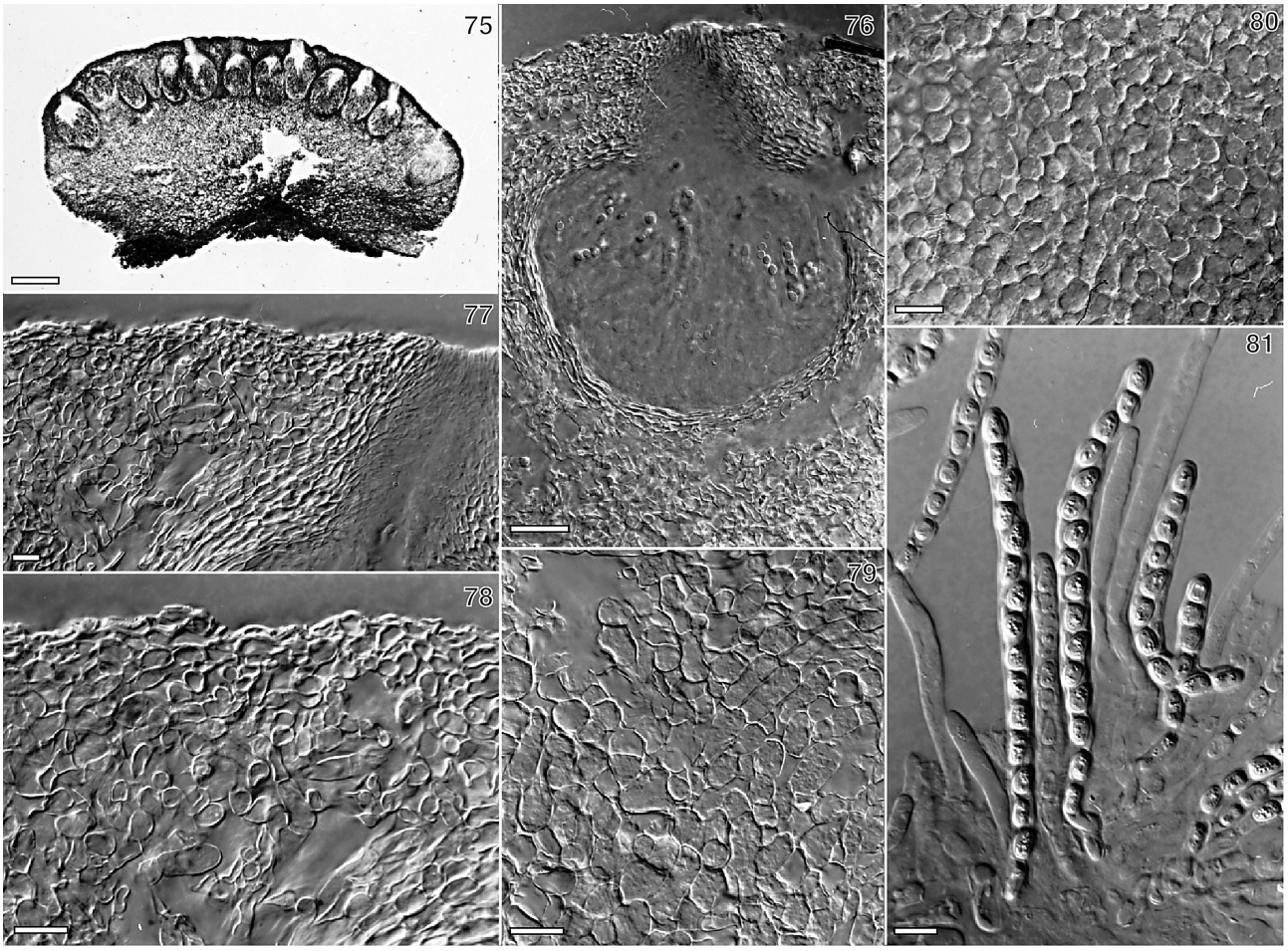
Stromata scattered, discrete, pulvinate, centrally attached with margins more or less free, 1.5–4.0 mm diam, light brown, not changing color in KOH. Stroma surface plane, smooth or slightly wrinkled, perithecial protuberances not evident, ostiolar openings appearing as viscid dots against a lighter background.



FIGS. 69–74. *Hypocrea semiorbis* anamorph. 69–72. Conidiophores and elongations. 73. Phialides and conidia. 74. Conidia. 69–71, 74 = DAOM 167636 (ex-type), 72, 73 = Isolate G.J.S. 99-108. Scale bars: 69–72 = 20 μm, 73, 74 = 10 μm.

Tissue of outermost layer of *textura angularis,* cells (9.0–)13.2–17.0(–30.0) μm diam (n = 25). Internal tissue of stroma of *textura angularis*. Perithecia subglobose (224–)268–287(–318) × (135–)160–177 (–202) μm (n = 25), ostiolar canal (59–)84–93(–109) μm long (n = 25). Asci cylindrical to narrowly clavate, (800–)102–107(–142) × (5.7–)7.5–7.7(–10.0) μm (n = 80). Ascospores uniseriate, part-ascospores hyaline, almost monomorphic and conical to wedge-shaped, distal part (4.7–)5.5–6.5(–8.5) × (3.5–)3.7–4.5(–5.2) μm, proximal part (4.7–)5.2–6.7(–9.0) × (2.7–)3.5–4.2(–5.2) μm (n = 90), finely but densely spinulose.

Colonies on CMD at 20 C after ca 2 wk forming pustules, pulvinate to subglobose, compact, 0.5–4.0 mm diam, with sterile hairs conspicuously arising from the entire pustule. Conidiophores typically comprising sterile hairs with short, broad fertile branches arising from the base of the sterile hairs. Sterile hairs conspicuous, straight to sinuous, septate, thin-walled, infrequently branched, subacute at the tip. Fertile branches progressively longer with distance from the tip of the sterile hair, the shortest branches comprising 2–3 cells, a few secondary branches arising from the lateral branches and also comprising 1–2 cells. Phialides densely disposed at the tips of and along the length of secondary branches and arranged in grape-like clusters, nearly doli-



FIGS. 75–81. *Hypocrea semiorbis* teleomorph. 75. Section of stroma. 76. Perithecium. 77, 78. Section of stroma with view of surface layers. 79, 80. Inner tissue below perithecia. 81. Asci and ascospores. 75, 77–79 = PDD 12751, 76 = PDD 12756, 80 = holotype, 81 = PDD 12755. Scale bars: 75 = 300 μm, 76 = 50 μm, 77–80 = 20 μm, 81 = 10 μm.

iform, (3.2–)4.5–6.5(–8.2) μm long, (2.7–)3.0–3.7(–4.5) μm at the widest point, (1.5–)2.0–3.0(–4.2) μm wide at the base, L/W (1.0–)1.3–2.0(–2.7) (n = 150), arising from a cell (2.7–)3.0–4.5(–6.0) μm wide (n = 150). Conidia green, oblong, smooth, 3.5–4.5(–7.0) × (1.7–)2.2–2.7(–3.0) μm, L/W (1.3–)1.6–1.7(–2.5) (n = 180). Chlamydospores typically not forming, intercalary, minute, (1.2–)1.5–2.5(–2.7) μm (n = 30).

Colonies on PDA at 25 C after ca 2 wk flat, white and dense, crustose, no pigment or distinctive odor noted. Colony radius after 3 d on PDA at 15 C: 12–23 mm, 20 C: 14–32 mm, 25 C: 18–30 mm, 30 C: 0 mm, and 35 C: 0 mm (n = 12). Colony radius after 3 d on SNA at 15 C: 5–8 mm, 20 C: 7–12 mm, 25 C: 10–17 mm, 30 C: 0–1 mm, and 35 C: 0 mm (n = 12).

Habitat.—Wood.

Known distribution.—Australia and New Zealand.

Holotype.—AUSTRALIA. Tasmania, on wood (K!)

Additional specimens examined.—NEW ZEALAND. HAWKES BAY: Upper Mohaka River, Kaimanawa Range, on *Nothofagus fusca* wood, May 1953, J. M. Dingley (PDD 12751, PDD 12755); Apr. 1953, J. M. Dingley (PDD 12756); Mohaka, on *Nothofagus* sp., J. M. Dingley (culture: DAOM 167636). NELSON: Nelson Lakes National Park, Lake Rotoiti, from South end of lake, trail along Travers River from Coldwater Hut, on branches of *Nothofagus* sp., 8 Sep 1999, G.J.S., S. Dodd (BPI 746666, culture: G.J.S. 99-108); on bark of *Nothofagus* sp., 8 Sep 1999, G.J.S., S. Dodd (BPI 746663, culture: G.J.S. 99-109).

Notes.—*Hypocrea semiorbis* and the teleomorph of *T. stromaticum* are the only known species with hyaline ascospores to have conidiophore apical elongations and green conidia. *Hypocrea pachybasioides* Doi, another species with hyaline ascospores, is morphologically similar to *H. semiorbis*. The anamorph of *H. pachybasioides* is *T. polysporum* (Link : Fr.) Rifai (see

Bissett 1991b, Gams and Bissett 1998), a species with white to yellow conidia. *Hypocrea semiorbis* can be distinguished mainly by its inability to grow at 30 C and by the fact that it has been found only in New Zealand and Australia. Based on RPB2 and EF-1α molecular sequence data, *H. semiorbis* is closely related to *T. oblongisporum* and *T. fertile*.

13. *Trichoderma spirale* Bissett, Can. J. Bot. 69: 2408. 1991. FIGS. 82–90, 158, 178

Colonies on CMD at 20 C after ca 2 wk with pustules forming around the periphery of the colony and a synanamorph forming abundantly in the aerial mycelium; no pigment or distinctive odor noted. Pustules pulvinate to subglobose, 0.5–2.0(–3.5) × 0.5–1.5(–3.0) mm diam (n = 15), compact, easily removed from the culture, with conspicuous sterile hairs arising from the entire pustule. Sterile hairs typically spiraled or sinuous, septate, thin-walled, infrequently branched, subacute. Conidiophores typically comprising a sterile hair and broad fertile branches arising from the base. Fertile branches progressively longer with distance from the tip of the sterile hair, the shortest branches comprising 2–3 cells; a few secondary branches arising from the fertile branches, comprising 1–2 cells; the secondary branches sometimes rebranching singly, directly from any of the branches, or arising in whorls at the tips of branches. Phialides arising in dense clusters, nearly doliiform, (3.2–)4.5–6.5(–8.2) μm long, (2.7–)3.0–3.7(–4.5) μm at the widest point, (1.5–)2.0–3.0(–4.2) μm wide at the base, L/W (1.0–)1.3–2.0(–2.7) (n = 60), arising from a cell (2.7–)3.0–4.5(–6.0) μm wide (n = 60). Conidia green, smooth, oblong to narrowly ellipsoidal, 3.5–4.5 × (2.0–)2.5–3.0(–3.7) μm, L/W (1.1–)1.4–1.5(–1.8) (n = 60). Chlamydospores typically abundant, intercalary, minute, globose to subglobose, (4.5–)7.0–15.0(–22.0) μm diam (n = 40). Synanamorphs abundant in the aerial mycelia, conidiophores mononematous, 50–100 μm long, bearing a single, terminal verticil or penicillus of phialides. Phialides cylindrical to slightly swollen in the middle, (6.7–)8.0–15.0(–22.0) μm long, (2.0–)2.5–3.5(–4.5) μm at the widest point, (1.5–)2.0–2.5(–3.5) μm at the base, L/W (2.3–)4.0–4.7(–8.9) (n = 60), arising from a cell (1.5–)2.2–3.2(–4.0) μm wide (n = 60), tip often flared and sometimes pigmented green. Conidia of synanamorph ellipsoidal, (3.4–)4.0–5.0(–6.2) × (2.2–)3.0–3.5(–4.0) μm, L/W (1.1–)1.3–1.4(–1.8) (n = 60).

Colonies on PDA at 25 after ca 2 wk with conidia forming in more or less distinct concentric rings, a yellow pigment tending to diffuse through the agar, no distinctive odor noted. Colony radius after 3 d on PDA at 15 C: 8–13 mm, 20 C: 29–40 mm, 25 C: 47–54 mm, 30 C: 60–70 mm, and 35 C: 0–4 mm (n = 6). Colony radius after 3 d on SNA at 15 C: 4–8 mm, 20 C: 20–35 mm, 25 C: 40–50 mm, 30 C: 51–63 mm, and 35 C: 0–13 mm (n = 6).

Habitat.—Soil, endophytic within trunks of *Theobroma cacao* trees.

Known distribution.—Cosmopolitan.

Cultures examined.—COSTA RICA PUNTARENAS: San Vito de Coto Brus, from premontane forest soil, 10 Apr 1979, J. Krug (DAOM 171919). THAILAND. Vicinity of Mekong R., from bamboo stand soil, 20 Feb 1982, H. B. Schieffer (DAOM 183974, ex-type).

Notes.—This species is morphologically similar to *T. fertile* in colony characteristics, conidiophore branching pattern and phialide distribution. *Trichoderma spirale* can be distinguished from *T. fertile* by the flexuous, unbranched, sterile apical conidiophore elongations and faster growth on PDA at 30 C; whereas *T. fertile* has straight conidiophore apical elongations, generally with one or two short, fertile apical branches, and slower growth on PDA. Based on RPB2 and EF-1α phylogenies, *T. spirale* is not closely related to any of the species studied.

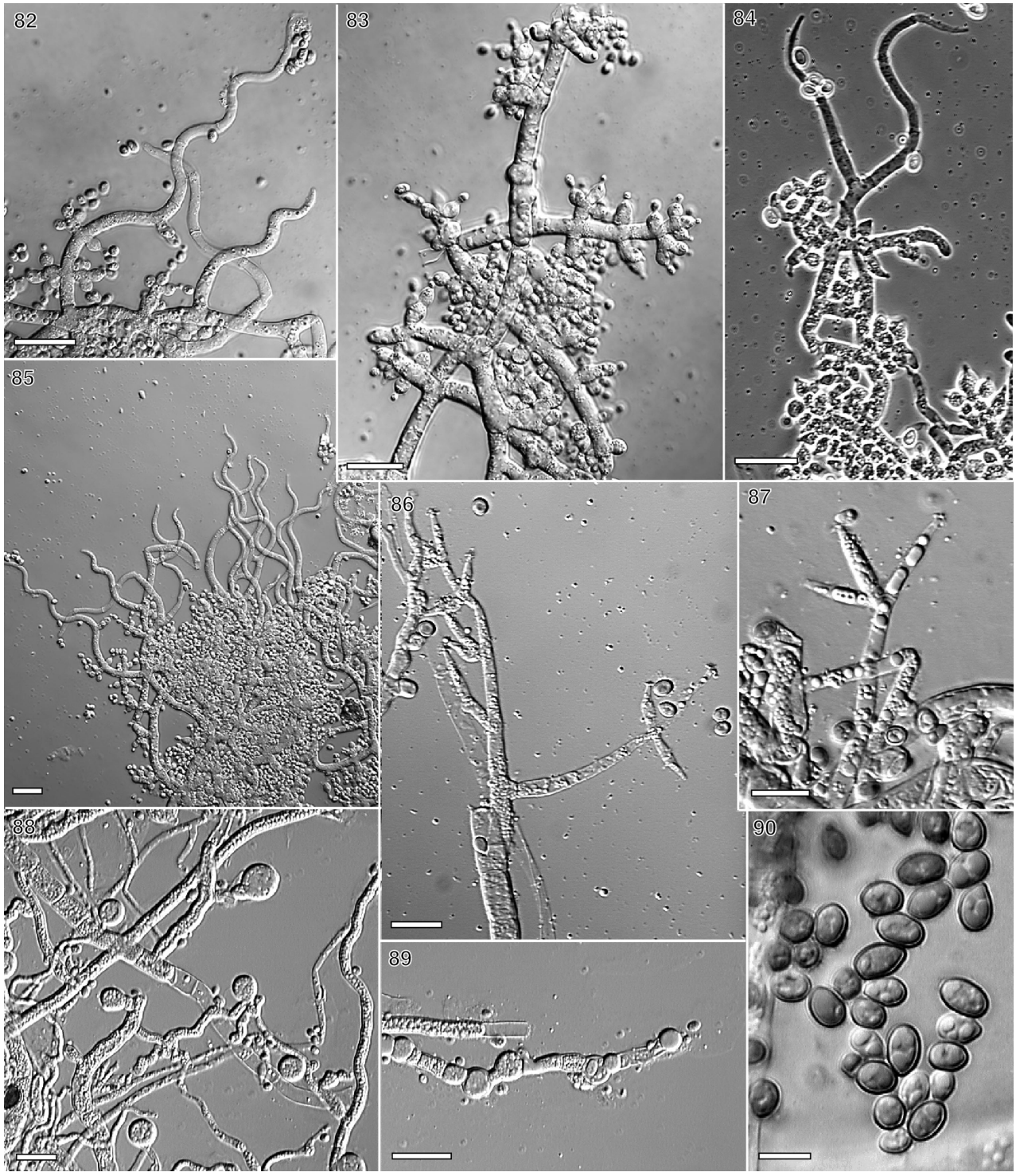
14. *Hypocrea strictipilosa* Chaverri et Samuels, sp. nov. FIGS. 91–113, 146, 147, 159–162, 179

Hypocreae cuneisporae similis sed ascosporis et conidiis minoribus, (2.7–)4.5–4.7(–8.0) × (2.2–)3.5–3.7(–5.2) μm, stromata glabra, KOH−, ascosporae minores, parte distali (4.2–)5.2–5.5(–7.0) × (3.2–)4.7–5.0(–6.0) μm, parte proximali (5.0–)5.7–6.2(–7.2) × (4.0–)4.7–5.2(–6.2) μm. Anamorphosis *Trichoderma strictipile* Bissett. Holotypus DAOM 172827.

Anamorph.—*Trichoderma strictipile* Bissett, Can. J. Bot. 69: 2410. 1991 (as "*strictipilis*", FIGS. 121–129).

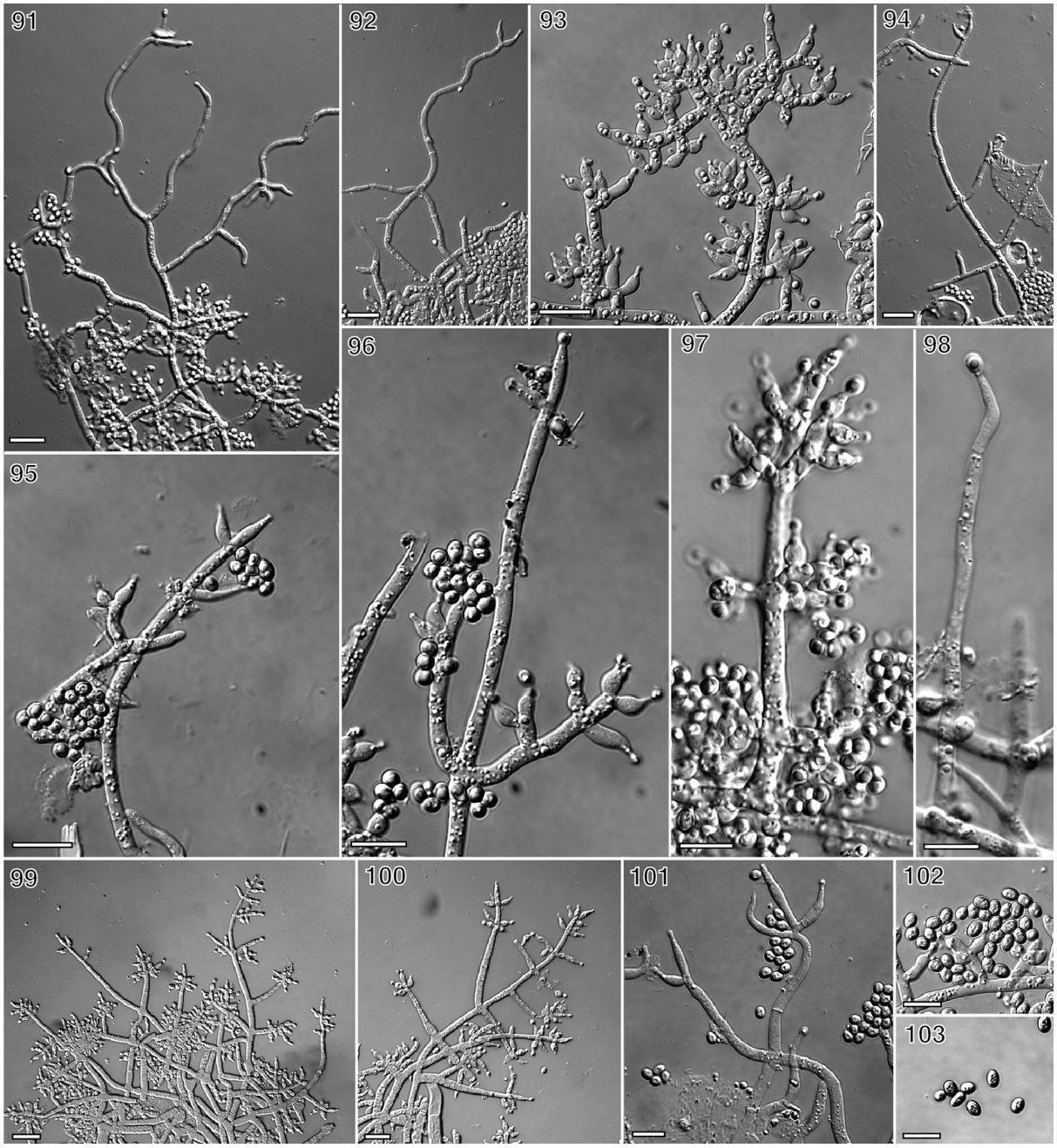
= *Trichoderma fasciculatum* Bissett, Can. J. Bot. 69: 2379. 1991 (FIGS. 18–21).

Stromata aggregated, in groups of 2–7, pulvinate, circular in outline, (0.5–)1.0–1.2(–2.0) mm diam (n = 90), (0.5–)0.6–0.7(–1.0) mm high (n = 40), broadly attached, surface smooth, with slight perithecial protuberances, yellowish white to light brown, not changing color in KOH, ostiolar openings obvious due to the green ascospores. Outermost layer of stroma composed of angular cells, hyaline, (3.7–)7.7–8.5(–17.0) μm diam (n = 150), walls (0.5–)0.7–1.0(–1.2) μm thick (n = 60). Tissue between perithecia and below the outermost layer composed of angular cells, hyaline, (3.5–)6.0–7.0(–20.7) μm diam (n = 95), walls 0.5–0.7(–1.0) μm thick (n = 45). Internal tissue below the perithecia of *textura angularis* to *epidermoidea* (3.2–)10.7–12.0(–25.7) μm diam (n = 140), walls (0.5–)0.7(–1.2) μm thick (n = 170). Perithecia immersed in stroma, generally closely aggregated or slightly separated, subglobose, (200–)237–252(–300) μm high, (88–)142–161(–217) μm wide



FIGS. 82–90. *Trichoderma spirale* (DAOM 183974, ex-type). 82–85. Conidiophores and elongations. 86, 87. Verticillium-like synanamorph. 88, 89. Chlamydospores. 90. Conidia. Scale bars: 75–78, 89 = 20 μm, 86, 87 = 10 μm, 88 = 15 μm, 90 = 5 μm.

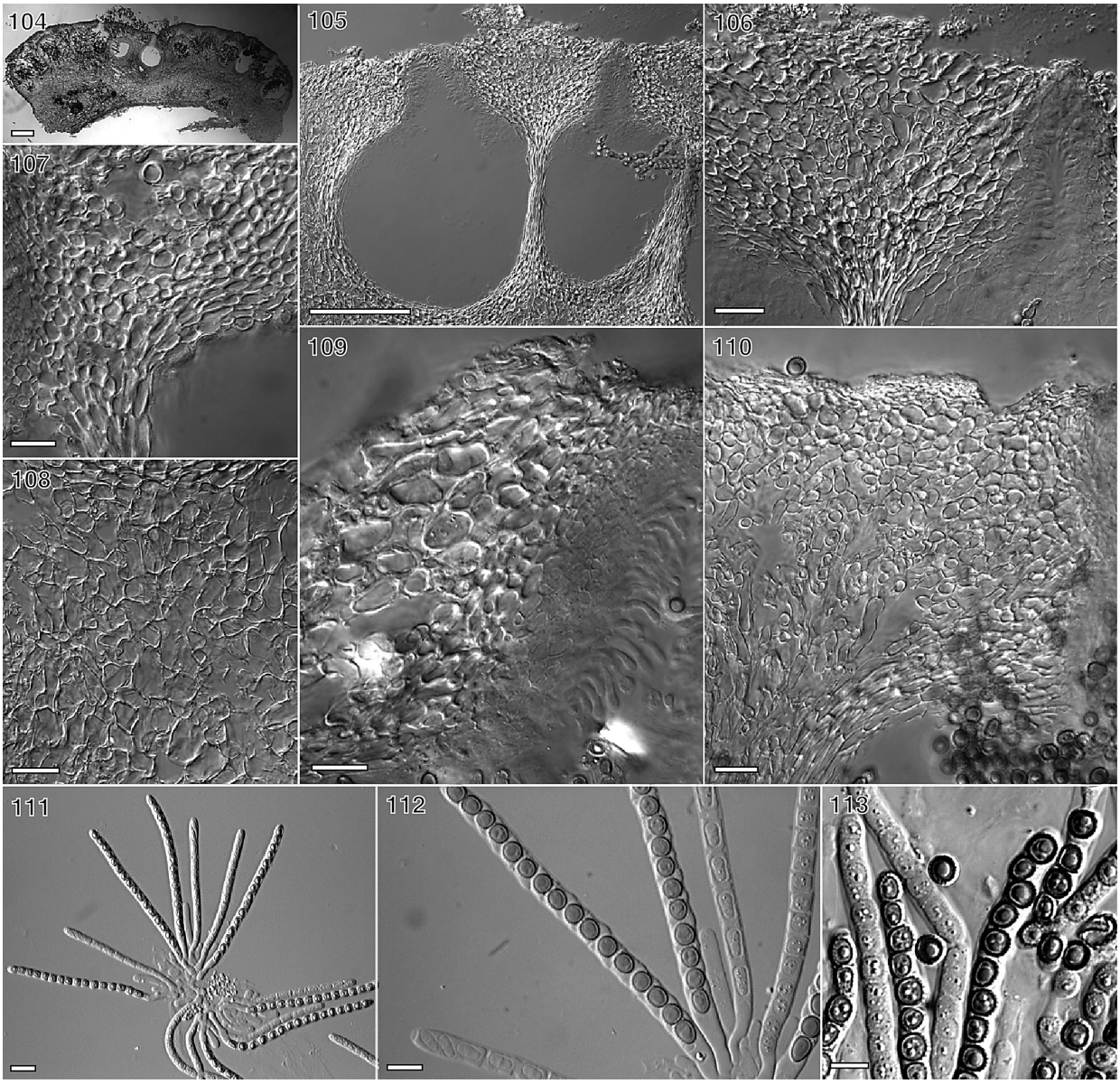
(n = 40), wall composed of compacted cells, becoming brownish in KOH, (9–)14–15(–22) μm thick (n = 65), ostiolar canal (53–)67–76(–109) μm long (n = 30). Asci cylindrical, uniseriate, (66–)92–98(–119) × (3.5–)5.7–6.0(–10.2) μm (n = 85). Part-ascospores green, warted, dimorphic, distal part globose to subglobose, (4.2–)5.2–5.5(–7.0) × (3.2–)4.7–5.0(–6.0) μm, proximal part generally cuneiform to sub-



FIGS. 91–103. *Trichoderma strictipile*. 91–100. Conidiophores and elongations. 101. Verticillium-like synanamorph. 102, 103. Conidia. 91, 92 = G.J.S. 89-114, 93, 94 = 89-115, 95 = G.J.S. 98-117, 96 = G.J.S. 00-171, 97, 98, 102 = DAOM 172827 (ex-type), 99, 100, 103 = DAOM 167646, 101 = G.J.S. 90-64. Scale bars: 91, 92, 94, 99, 100 = 20 μm, 93, 95 = 15 μm, 96–98, 101–103 = 10 μm.

globose, (5.0–)5.7–6.2(–7.2) × (4.0–)4.7–5.2(–6.2) μm (n = 175).

Colonies on CMD at 20 C after ca 1 wk flat with tufts pulvinate to irregular, loose to compact, 1–3 mm diam, formed around the periphery of the colony. Conidiophores typically consisting of a central axis with lateral branches arising singly or in pairs, the lateral branches increasing in length further from the tip of the main axis. The lateral branches producing a terminal whorl of phialides and, often, phialides arising from below the tip of the lateral branch or the main axis and, just below the tip, phialides arising directly from the main axis either singly or in a whorls of 2–4. Conidiophore elongations long-branched or unbranched, straight or flexuous, sterile or fertile, when fertile with 1 or 2 phialides.



FIGS. 104–113. *Hypocrea strictipilosa*. 104. Section of stroma. 105. Perithecia. 106. Section of stroma with view of surface layers. 107. Tissue between perithecia. 108. Inner tissue below perithecia. 109. Ostiolar canal and stroma surface layer. 110. Section of stroma with view of surface layers. 111. Asci. 112, 113. Asci and ascospores. 104, 110, 113 = G.J.S. 96-190, 105, 111 = DAOM 172827 (holotype), 106, 108 = G.J.S. 96-189, 107, 109 = G.J.S. 96-130, 112 = G.J.S. 91-126. Scale bars: 104 = 250 μm, 105 = 150 μm, 106–108, 110, 111 = 15 μm, 109, 112, 113 = 10 μm.

Phialides mainly arising in divergent whorls, less frequently singly and then at or near the tip of the main axis, somewhat swollen and ampulliform, straight, (4.0–)7.2–7.7(–21.7) μm long, (2.0–)4.0–4.2(–5.7) μm at the widest point, (1.5–)2.7–3.0(–4.2) μm at the base, L/W (1.0–)1.8–2.0(–7.4) (n = 450); phialides from conidiophore apical elongations (4.7–)13.0–14.5(–31.0) μm long, (2.1–)3.5–3.7(–5.0) μm at the widest point, (1.5–)2.7–3.0(–4.7) μm at the base, L/W (1.3–)3.8–4.3(–10.7) (n = 180), arising from a cell (2.5–)4.0–4.2(–6.5) μm wide (n = 325). Conidia arising from short phialides green, ellipsoidal, smooth, (2.7–)4.5–4.7(–8.0) × (2.2–)3.5–3.7(–5.2) μm, L/W (0.9–)1.3(–2.2) (n = 500); conidia from conidiophore elongations (3.3–)5.0–5.7(–11.5) × (2.5–)3.5–4.0(–5.5) μm, L/W (1.0–)1.3–1.5(–2.2) (n = 75). No chlamydospores observed.

Colonies on PDA at 25 C after 1 wk flat, with some aerial mycelium, conidia formed from the point of inoculum outwards in concentric rings; diffusing yellow pigment not noted; no distinctive odor detected. Colony radius after 3 d on PDA at 15 C: 4–20 mm,

20 C: 10–44 mm, 25 C: 23–62 mm, 30 C: 36–62 mm, and 35 C: 0–10 mm (n = 30). Colony radius after 3 d on SNA at 15 C: 4–16 mm, 20 C: 15–41 mm, 25 C: 23–61 mm, 30 C: 18–48 mm, and 35 C: 0–10 mm (n = 30).

Habitat.—On ascomycetous and basidiomycetous fungi, and wood.

Known distribution.—U.S.A. (Indiana, Maryland, New York, Pennsylvania), Canada (Quebec), northeastern Europe (Denmark, Estonia, Germany, France).

Holotype.—CANADA. QUEBEC: Montreal, on rotting log, 20 Sep 1979, G. P. White (DAOM 172827; culture: DAOM 172827, ex-type isolate of *T. strictipile*).

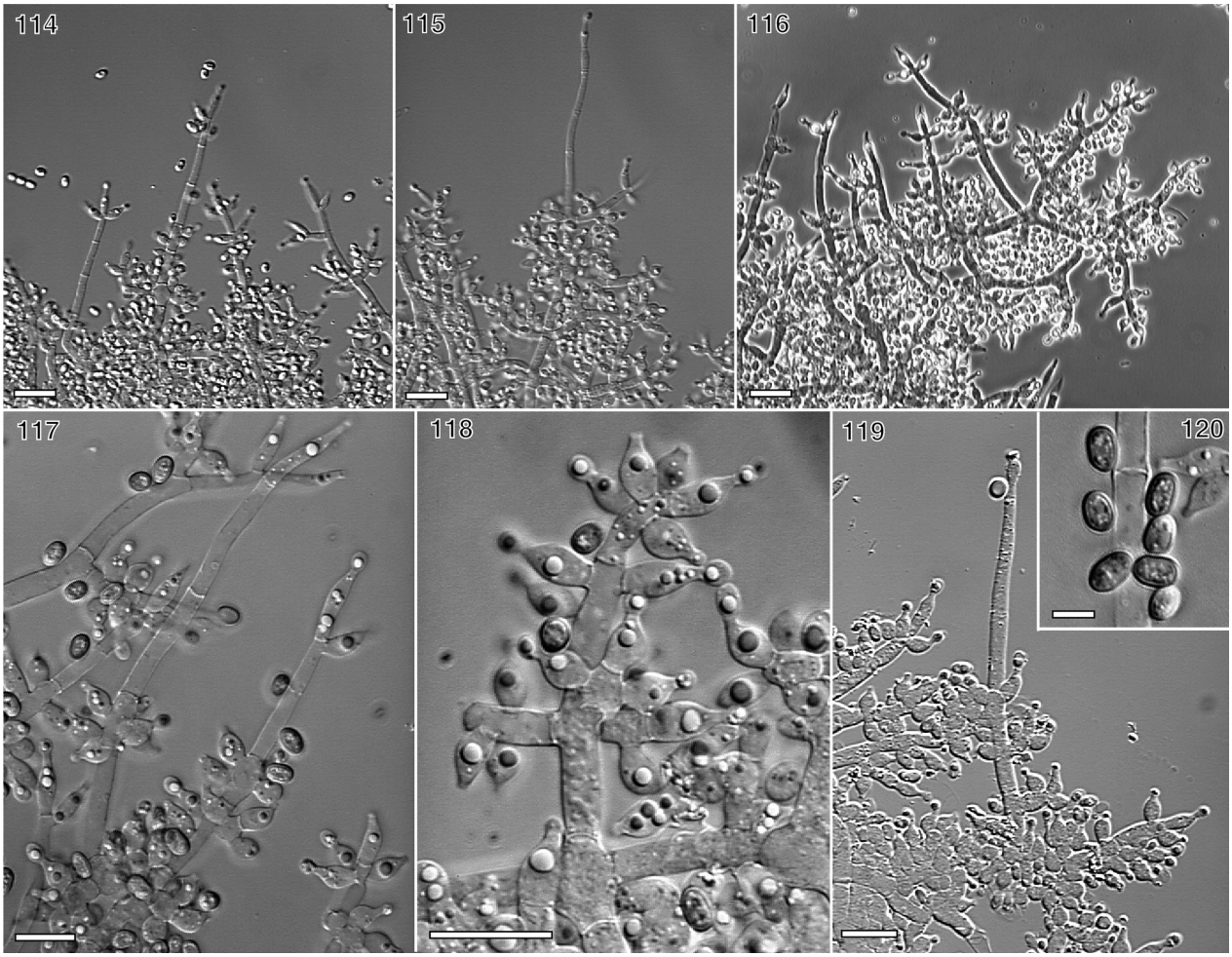
Additional cultures and specimens examined.—CANADA. QUEBEC: Lacolle, from maple forest soil, May 1976, P. Widden (culture: DAOM 167062 as *T. strictipilis*). DENMARK. RINKANAS SKOV: near Grasten, Jutland, on wood, 29 Sep 1978, H. Dissing (NY, culture: C.T.R. 78-201). ESTONIA. TARTU: Tahtrere Forest, on wood of deciduous tree, 13 Apr 1996, K. Põldmaa (TAA 161841 = BPI 744576, culture: G.J.S. 96-130). FRANCE. PYRÉNÉES ATLANTIQUES: Bois D'Evieu, Ain, on wood, 30 Oct 1994, J. F. Magni (BPI 744476, culture: G.J.S 94-97). GERMANY. SAARLAND: Naturpark Saar-Hundsrück, between Leisel and Schollen, alt. 600 m, 49°40′N 7°10′W, on decorticated wood of *Picea* sp. decorticated wood, 13 Oct 1998, G.J.S., H-J. Schroers (BPI 748291, culture: G.J.S. 98-110); on decorticated wood, G.J.S. (BPI 748294, culture: G.J.S. 98-113); on decorticated wood, 13 Oct 1998, G.J.S., H-J. Schroers (BPI 748298, culture: G.J.S. 98-117). RUSSIA. Moscow region, 50 km south of Moscow, on *Betula* sp. wood, 25 Oct 2000, A. Alexandrova (BPI 842023, culture: G.J.S. 00-170); 10 km northeast of Moscow, on *Alnus* sp. wood, 17 Oct 2000, A. Alexandrova (BPI 842024, culture: G.J.S. 00-171). UNITED STATES. INDIANA: Porter County, Indiana Dunes National Lakeshore, in hardwood area, on bark, 19 Aug 1996, J. Murphy (BPI 744525, culture: G.J.S. 96-190); on hymenium of *Phellinus ferruginosus*, 19 Aug 1996, J. Murphy (BPI 744524, culture: G.J.S. 96-189). MARYLAND. Garret County, 5 miles north of Barton, Little Savage River Ravine, 23 Sep 1989, G.J.S., C.T.R., W. R. Buck, R. C. Harris (NY, culture: G.J.S. 89-114); on decorticated wood, G.J.S. (NY, culture: G.J.S. 89-115). NEW YORK: Brockton, Route 680, Chautauqua County, on wood, 2 Sep 1979, C.T.R. (BPI 631330, culture: C.T.R. 77-149); Dutchess County, east side of Pawling, Pawling Nature Reserve, Nature Conservancy, on bark of *Pinus* sp., 6–8 Oct 1990, G.J.S. & C. T. Rogerson (BPI 1107141, culture: G.J.S. 90-64). PENNSYLVANIA: Mercer County, Grove City Community Park, on decorticated wood, 12 Sep 1998, G.J.S. (BPI 748275, culture: G.J.S. 98-91).

Notes.—*Hypocrea strictipilosa* resembles *H. cuneispora* and *H. aureoviridis* f. *macrospora* in stroma morphology and large ascospore size. *Hypocrea cuneispora* has significantly larger conidia and ascospores, and the stroma of *H. strictipilosa* is smooth and KOH−, whereas in *H. cuneispora* it is roughened and KOH+. RPB2 and EF-1α gene genealogies show that *H. strictipilosa* is closely related to *H. cuneispora* and *T. longipile*. *Hypocrea strictipilosa* also is morphologically similar to *H. a.* f. *macrospora*, in that both have yellowish stromata and large ascospores. *Hypocrea strictipilosa* can be distinguished from *H. a.* f. *macrospora* mainly by the conidial L/W, 1.2–1.3 in *H. strictipilosa* and ca 1.7 in *H. a.* f. *macrospora*. Because it was not possible to examine the type culture of *H. a.* f. *macrospora* we were not able to determine if *H. strictipilosa* is a synonym, but the differences in conidial measurements and L/W suggest they are not. *Hypocrea/Trichoderma strictipile* is closely related to *T. longipile* and *H. cuneispora* based on RPB2 and EF-1α gene genealogies.

15. *Trichoderma strigosum* Bissett, Can. J. Bot. 69: 2411. 1991. FIGS. 114–120, 164, 180

Colonies on CMD at 20 C after ca 2 wk with conidia forming well around the periphery of the colony in discrete to ill-defined pustules or extensive flat areas. A weak to strong sweet, coconut odor detected. No diffusing pigment observed. Pustules pulvinate to hemispherical, 0.5–1.0 mm diam or irregular in shape and to 5 mm in greatest dimension, or conidial production continuous and not pustulate. Conidiophores formed in more or less discrete pustules comprising a more or less distinct central axis and fertile to the tip or with a 30–90 μm long, stiff, thin-walled, septate, unbranched elongation terminating in 1–3 phialides; conidiophores 3.5–6.5 μm wide at the base of the elongated portion; fertile branches arising at or near 90° with respect to the elongated portion and producing phialides directly or producing secondary branches at 90°, each secondary branch producing phialides along its length and at the tip. Sterile hairs sometimes present, identical to the fertile extensions but lacking phialides, acute at the tip, arising from the entire pustule. Phialides ampulliform, conspicuously larger in the middle or not, (4–)5–8(–11) μm long, (1.7–)2.7–3.7(–4.5) μm at the widest point, (1.2–)1.7–2.7(–3.7) μm at the base, L/W (1.3–)2.2–3.8(–5.0) (n = 60), arising from a cell (2.0–)2.5–3.5(–5.0) μm wide (n = 60). Conidia green to gray-green, smooth, oblong, (3.2–)3.5–4.5(–5.5) × (1.7–)2.0–3.0(–3.2) μm, L/W (1.2–)1.5–1.6(–1.8) (n = 60). Chlamydospores not observed.

Colonies on PDA at 25 C after ca 1 wk with conidia forming in a dense central disk with conidial production just beginning elsewhere; no pigmentation of the agar; strong coconut odor detected. Colony radius after 3 d on PDA at 15 C: 9–12 mm, 20 C: 30–38 mm, 25 C: 43–53 mm, 30 C: 36–70 mm, and 35 C: 0–5 mm (n = 6). Colony radius after 3 d on SNA



FIGS. 114–120. *Trichoderma strigosum.* 114–117, 119. Conidiophores and elongations. 118. Phialides. 120. Conidia. 114–118, 120 = DAOM 166121 (ex-type), 119 = DAOM 166140. Scale bars: 114–116 = 15 μm, 117–119 = 10 μm, 120 = 5 μm.

at 15 C: 3–5 mm, 20 C: 13–18 mm, 25 C: 30–35 mm, 30 C: 33–45 mm, and 35 C: 3–10 mm (n = 6).

Habitat.—Soil.

Known distribution.—U.S.A. (North Carolina) and Brazil.

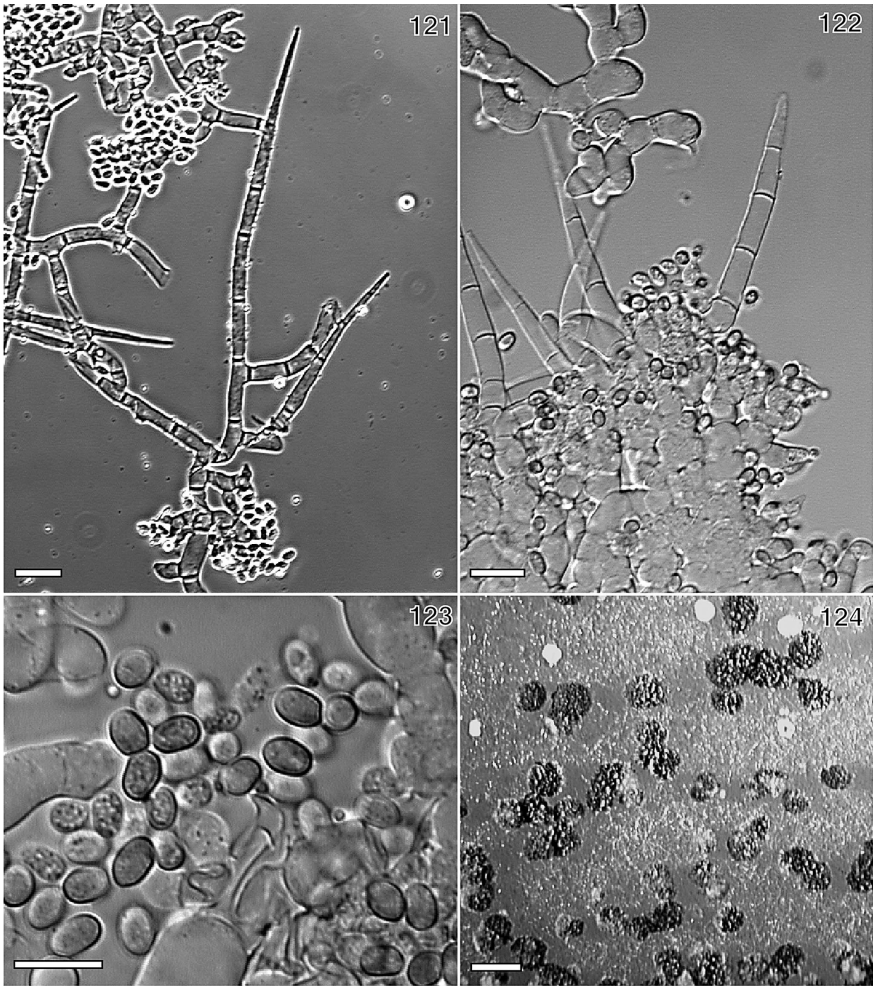
Cultures examined.—BRAZIL. PARÁ: Belém, EMBRAPA, isolated from trunk of live *Theobroma* sp., date unknown, H. C. Evans DIS 173k (BPI as DIS 173k). UNITED STATES. NORTH CAROLINA: Hoffman forest, from forest soil, 20 Feb 1969, R. M. Danielson (DAOM 166140 = ATCC 28031); Singletary Lake, from hardwood forest soil, May 1969, R. M. Danielson (DAOM 166121 = ATCC 28042, ex-type).

Notes.—This species is related to *T. hamatum* and *T. pubescens*, morphologically and phylogenetically (based on RPB2 and EF 1-α sequence data). Molecular data also place *T. strigosum* as a sister species to *H. rufa* (see also Lieckfeldt et al 1998b). *Trichoderma strigosum* can be distinguished from its closest relatives by the conidiophore apical elongations, which are straight and branched near the fertile part of the conidiophore. Conidia of *T. strigosum* are striking because of the large L/W ratio and nearly parallel sides.

16. *Trichoderma stromaticum* Samuels & Pardo-Schultheiss, Mycol. Res. 104: 762. 2000.
FIGS. 121–124, 165, 166, 181

Notes.—Colony radius after 3 d on PDA at 20 C: 21–34 mm, 25 C: 45–56 mm, 30 C: 13–28 mm, and 35 C: 0 mm (n = 8). Colony radius after 3 d on SNA at 20 C: 2–10 mm, 25 C: 10–18 mm, 30 C: 0–15 mm, and 35 C: 0 mm (n = 8). This species is easily recognized by the compact pustules that can be easily dislodged from the agar surface, formed of vesicular to pseudoparenchymatous cells and arranged in chains that branch near the surface of the pustule. This species was described in detail in Samuels et al (2000).

Cultures examined.—BRAZIL. BAHIA: Reserva UNA, on *Theobroma cacao* pods infected with *Crinipellis perniciosa*, Mar 2002, P. Hebbar (P.C. 209). PARÁ: on artificially inoculated *C. perniciosa* on *T. cacao*, Aug 2000, G.J.S. (G.J.S. 00-



FIGS. 121–124. *Trichoderma stromaticum.* 121, 122. Conidiophores and elongations. 123. Conidia. 124. Conidiogenous pustules. 121–123 = G.J.S. 00-02, 1124 = G.J.S. 00-91. Scale bars: 121, 123 = 10 μm, 122 = 20 μm, 124 = 2 mm.

91). COLOMBIA. On *T. cacao* pods infected with *C. perniciosa*, P. Hebbar 406 (G.J.S. 00-02).

17. *Hypocrea surrotunda* Chaverri et Samuels, sp. nov. FIGS. 125–132, 145, 163, 182

Hypocreae cremeae similis sed ascosporis minoribus, viridibus, parte distali (4.2–)5.0–5.5(–6.0) × (4.2–)5.0–5.5(–6.0) μm, parte proximali, (3.7–)4.7–5.2(–6.0) × (4.0–)4.5–4.7(–5.2) μm. Anamorphosis *Trichoderma* sp. Phialidis (6.5–)8.5–9.5(–13.0) × (3.0–)3.7–4.0(–4.5) μm; conidii viridia, glabra, (4.2–)4.5–5.0(–5.5) × (3.2–)3.7–4.0(–4.2) μm, ratio longitudo/crassitudo (1.1–)1.2–1.3(–1.6). Incrementum in agaro "PDA" dicto post 72 h tardum, 15 C = 9–14 mm, 20 C = 18–20 mm, 25 C = 26–32 mm, 30 C = 20–22 mm, 35 C = 0 mm. Holotypus in NY.

Anamorph.—Trichoderma sp.

Stromata scattered, solitary, pulvinate almost spherical, circular in outline and in transverse section, 1.5–2.6(–2.8) mm diam (n = 6), 873–949 μm high (n = 5), base constricted, surface smooth, with no perithecial protuberances, light yellow, KOH−, ostiolar openings obvious due to the green ascospores. Outermost layer of stroma composed of angular cells, hyaline, (6.2–)7.7–9.0(–12.5) μm diam (n = 30), walls 0.5–0.7 μm thick (n = 15). Tissue between perithecia and below the outermost layer composed of hyaline intertwined hyphae to *textura epidermoidea,* cells (6.7–)9.5–13.5(–18.2) μm diam (n = 30), walls (0.5–)0.7–1.0 μm thick (n = 15). Internal tissue below perithecia of *textura angularis,* hyaline, cells (11.2–)15.5–20.2(–37.2) μm diam (n = 30), walls (0.5–)0.7–1.0(–1.5) μm thick (n = 15). Perithecia immersed in stroma, generally closely aggregated or slightly separated, subglobose, 234–244 μm high, 170–207 μm diam (n = 5), wall composed of compacted cells, KOH−, 16.8–22.7(–24.9) μm thick (n = 10); ostiolar canal 56.5–61.9 μm long (n = 5). Asci cylindrical, (92–)96–110(–120) × (4.5–)5.0–5.7(–6.5) μm (n = 15). Part-ascospores green, warted, dimorphic, distal part globose to subglobose (4.2–)5.0–5.5(–6.0) × (4.2–)5.0–5.5(–6.0) μm, proximal part

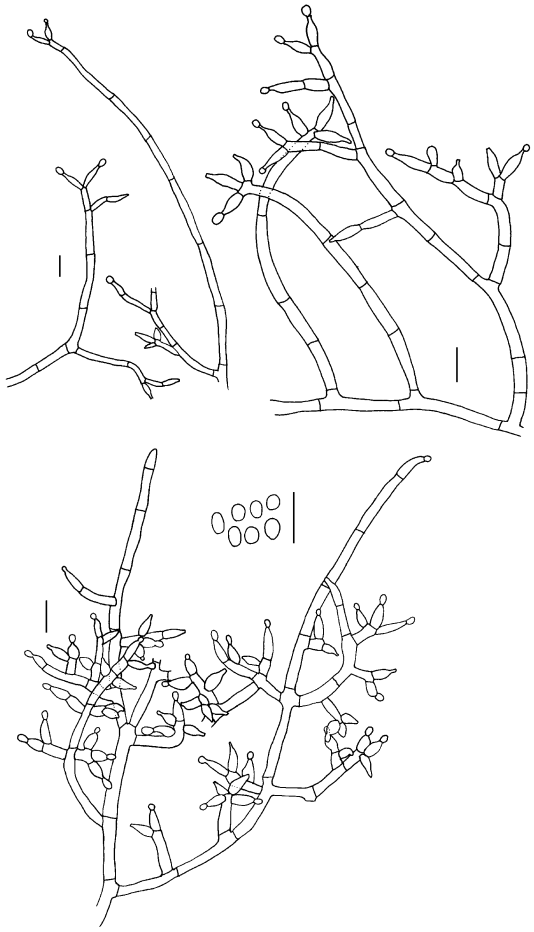


FIG. 125. *Hypocrea surrotunda* anamorph (holotype). Scale bars = 10 μm.

cuneiform, (3.7–)4.7–5.2(–6.0) × (4.0–)4.5–4.7(–5.2) μm (n = 30).

Colonies on CMD at 20 C after ca 1 wk flat, with compact tufts 2–3 mm diam (n = 10) forming near the edge of the plate, conidia produced after ca 2 wk, no distinctive odor, no pigmentation of the agar. Branching pattern of the conidiophores irregular with phialides arising from the subtending hyphae at wide angles. Phialides ampulliform, formed in whorls of (1–)2–3(–4), (6.5–)8.5–9.5(–13.0) μm long, (3.0–)3.7–4.0(–4.5) μm at the widest point, (1.5–)2.5–2.7(–3.2) μm at the base, L/W (1.6–)2.2–2.6(–3.6) (n = 30), elongations of the conidiophore common, fertile or sterile, terminating in ampulliform phialides; subtending hyphae cylindrical, (3.0–)3.5–4.0(–5.0) μm wide (n = 15). A synanamorph with longer branches and 1 or 2 phialides sometimes is observed. Conidia green, smooth, ellipsoidal to oblong, (4.2–)4.5–5.0(–5.5) × (3.2–)3.7–4.0(–4.2) μm, L/W (1.1–)1.2–1.3(–1.6) (n = 30). No chlamydospores observed.

Colonies on PDA at 25 C after ca 1 wk flat, with some aerial mycelium, conidia formed from the point of inoculum outward in concentric rings, the outermost ring with a feathery appearance, sectoring or radiating lines formed from the point of inoculum, conidia formed after ca 4 d, no pigmentation of the agar, no distinctive odor. Colony radius after 3 d on PDA at 15 C: 9–14 mm, 20 C: 18–20 mm, 25 C: 26–32 mm, 30 C: 20–22 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 2–7 mm, 20 C: 6–14 mm, 25 C: 12–24 mm, 30 C: 19–26 mm, and 35 C: 0 mm (n = 3).

Etymology.—Shortened from Latin *supra rotundus* meaning "rounded above," alluding to the shape of the stromata.

Habitat.—On decorticated wood, probably growing on another fungus.

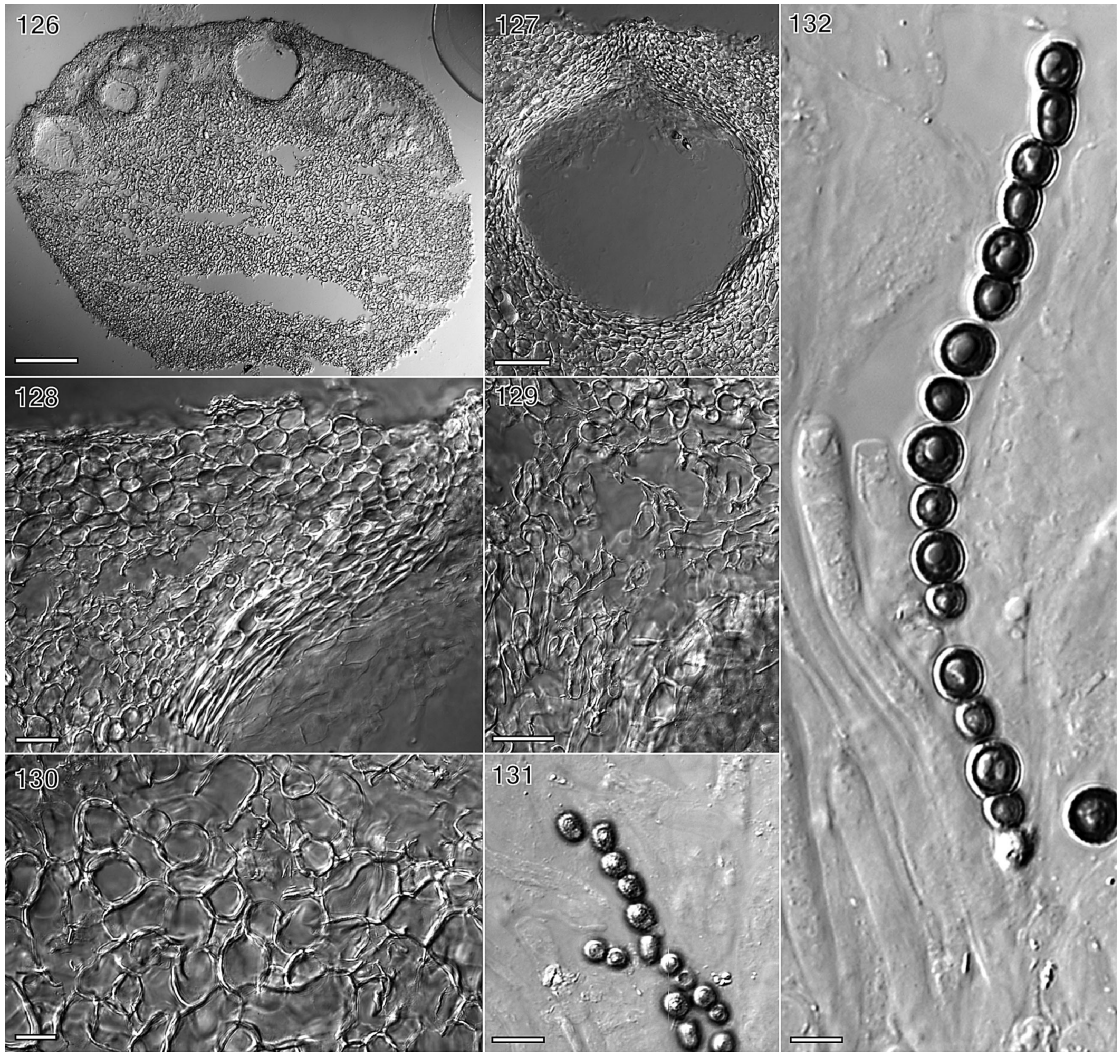
Known distribution.—U.S.A. (Connecticut).

Holotype.—UNITED STATES. CONNECTICUT: Fairfield County, Weston, Devil's Glen Conservancy, on decorticated wood, Nov 1988, S. Stein (NY; cultures: G.J.S. 88-73 = ATCC MYA-2865 = CBS 111145 = DAOM 231315).

Notes.—This species resembles *H. cremea* in stroma morphology and anamorph. *Hypocrea surrotunda* is distinguished by the slightly smaller ascospores and significantly slower growth on PDA and SNA. In addition, the optimum growth temperature is 25 C for *H. surrotunda* and 30 C for *H. cremea*. RPB2 and EF-1α gene genealogies show that *H. surrotunda* and *H. cremea* are closely related.

18. *Trichoderma tomentosum* Bissett, Can. J. Bot. 69: 2412. 1991. FIGS. 133–139, 167, 183

Colonies on CMD at 20 C after ca 1 wk with conidiophores and conidia forming pustules in a narrow band around the edge of the colony and a synanamorph forming abundantly in the scantily formed aerial mycelium. Pustules 0.5–1.5(–2.3) mm diam, pulvinate and conspicuously hairy, very dense and conidia appearing to be held in waxy matrix, easily removed from the agar, conidia not easily dispersing in 3% KOH. Conidiophores typically with an unbranched or infrequently branched sterile hair from the base of which short, broad lateral branches arise at right angles, rebranching at right angles to produce secondary fertile branches with clusters of phialides arising from the primary and secondary branches. Sterile hairs spiraled, infrequently branched, appearing roughened when viewed with stereomicroscope, thin-walled, septate, and acute at the tip. Phialides tending to be short and broad, almost ovoidal with a distinct neck, (3.7–)4.5–5.0(–6.2) μm long, (2.2–)3.0–3.2(–4.0) μm at the widest point, (1.0–)2.0–2.5(–3.5) μm at the



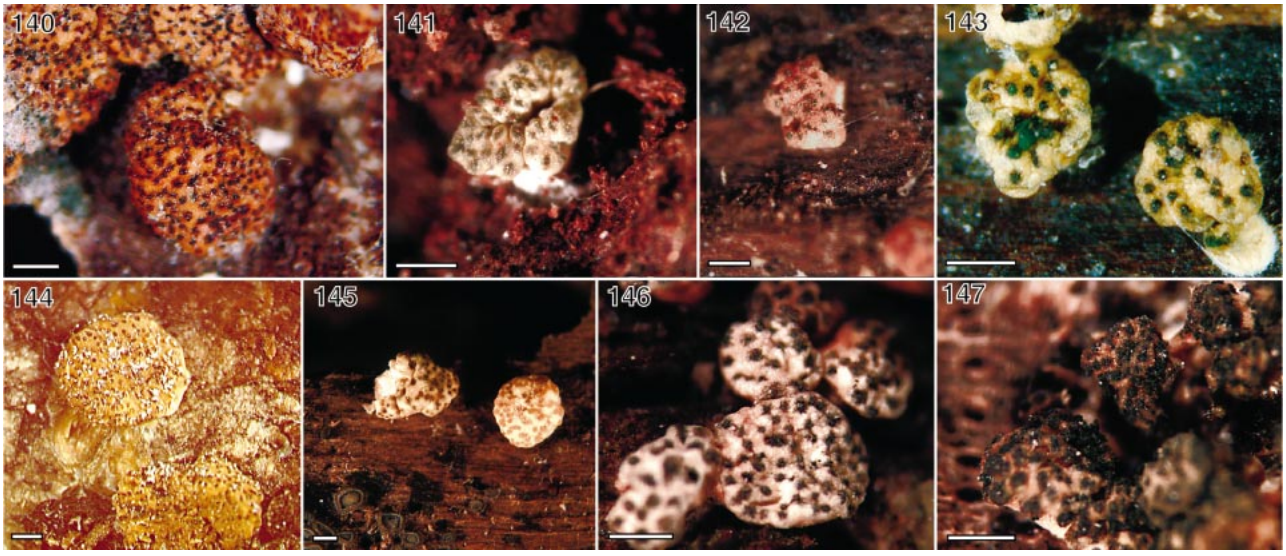
FIGS. 126–132. *Hypocrea surrotunda* teleomorph (holotype). 126. Section of stroma. 127. Perithecium. 128. Stroma surface layer. 129. Tissue between perithecia. 130. Inner tissue below perithecia. 131. Ascospores. 132. Asci and ascospores. Scale bars: 126 = 200 μm, 127 = 50 μm, 128, 130, 131 = 10 μm, 129 = 15 μm, 132 = 5 μm.

base, L/W (1.1–)1.5–1.6(–2.0), subtending cells (2.5–)3.2–3.5(–4.2) μm wide (n = 30), forming in dense, grape-like clusters at the tips of fertile branches. Conidia gray-green, smooth, broadly ellipsoidal, (2.7–)3.2–3.5(–3.7) × (2.0–)2.2–2.5(–2.7) μm, L/W (1.2–)1.4(–1.6) (n = 30). Chlamydospores scattered, globose to subglobose, (5.0–)6.5–7.2(–9.7) μm diam (n = 30), terminal or intercalary. Synanamorphs forming abundantly in the aerial mycelium, 65–100 μm long, verticillately- or penicillately-branched at the tip. Phialides tending to be swollen in the middle, (5.7–)8.5–10.0(–14.0) μm long, (2.2–)2.5–2.7(–3.2) μm wide in the middle, (1.0–)1.7–2.2(–3.2) μm at the base, L/W (2.2–)3.2–3.7(–5.5) (n = 30). Conidia held in a drop of clear, green, watery liquid at the tip of each phialide, smooth, ellipsoidal, (3.0–)3.5–3.7(–4.2) × (2.2–)2.5–2.7(–3.2) μm, L/W (1.0–)1.3–1.4(–1.6) (n = 30).

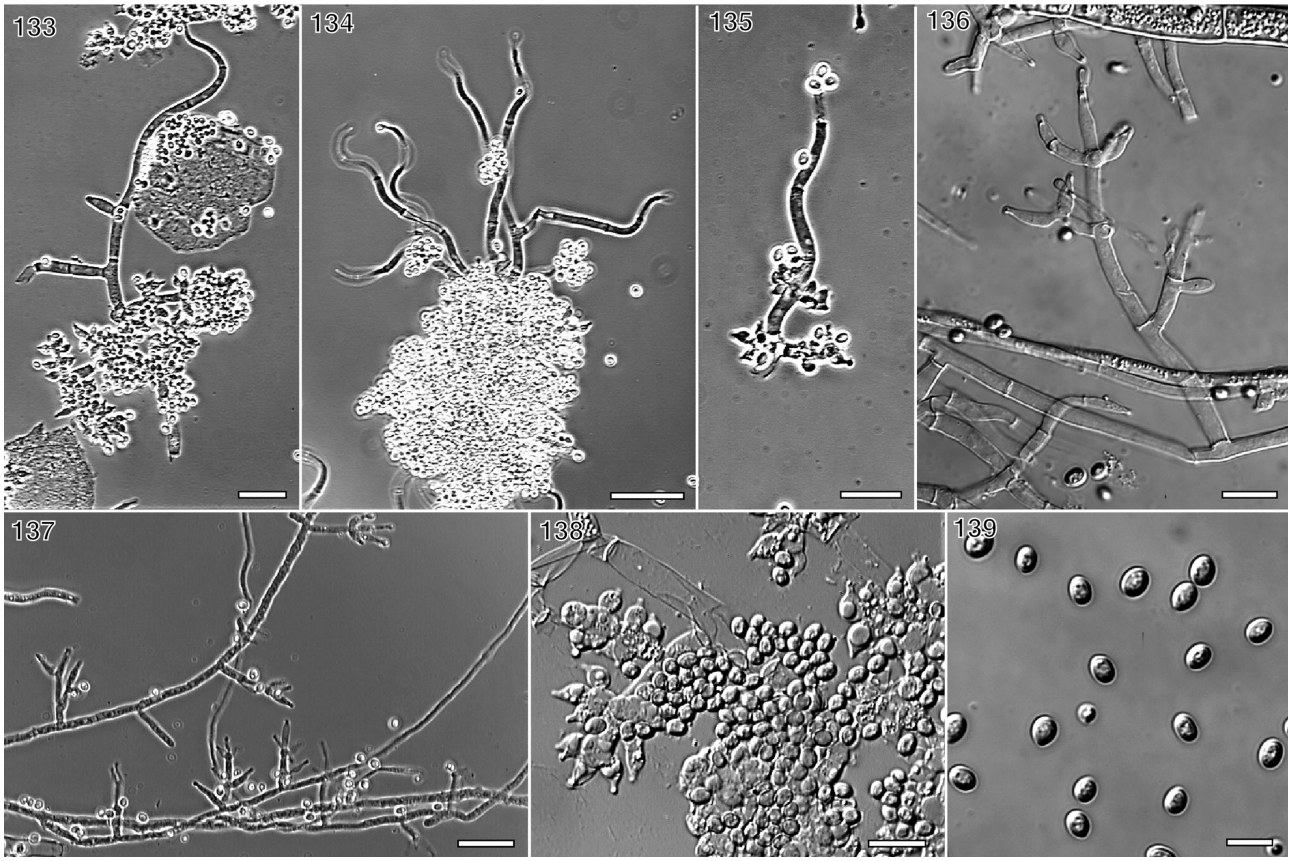
Colonies on PDA at 25 C after ca 1 wk, with conidia forming in pronounced concentric rings, no diffusing pigment or distinctive odor noted. Colony radius after 3 d on PDA at 15 C: 10–11 mm, 20 C: 25–30 mm, 25 C: 40–45 mm, 30 C: 46–50 mm, and 35 C: 0 mm (n = 3). Colony radius after 3 d on SNA at 15 C: 8–10 mm, 20 C: 20–25 mm, 25 C: 30–35 mm, 30 C: 35–42 mm, and 35 C: 0–1 mm (n = 3).

Notes.—*Trichoderma tomentosum* can be distinguished by the small conidia, short phialides and conidiophore apical elongations that are sterile, long and flexuous. Based on the RPB2 and EF-1α genealogies produced in this study, *T. tomentosum* is a sister species to *H. atrogelatinosa* and closely related to *T. aggressivum* and *T. harzianum*.

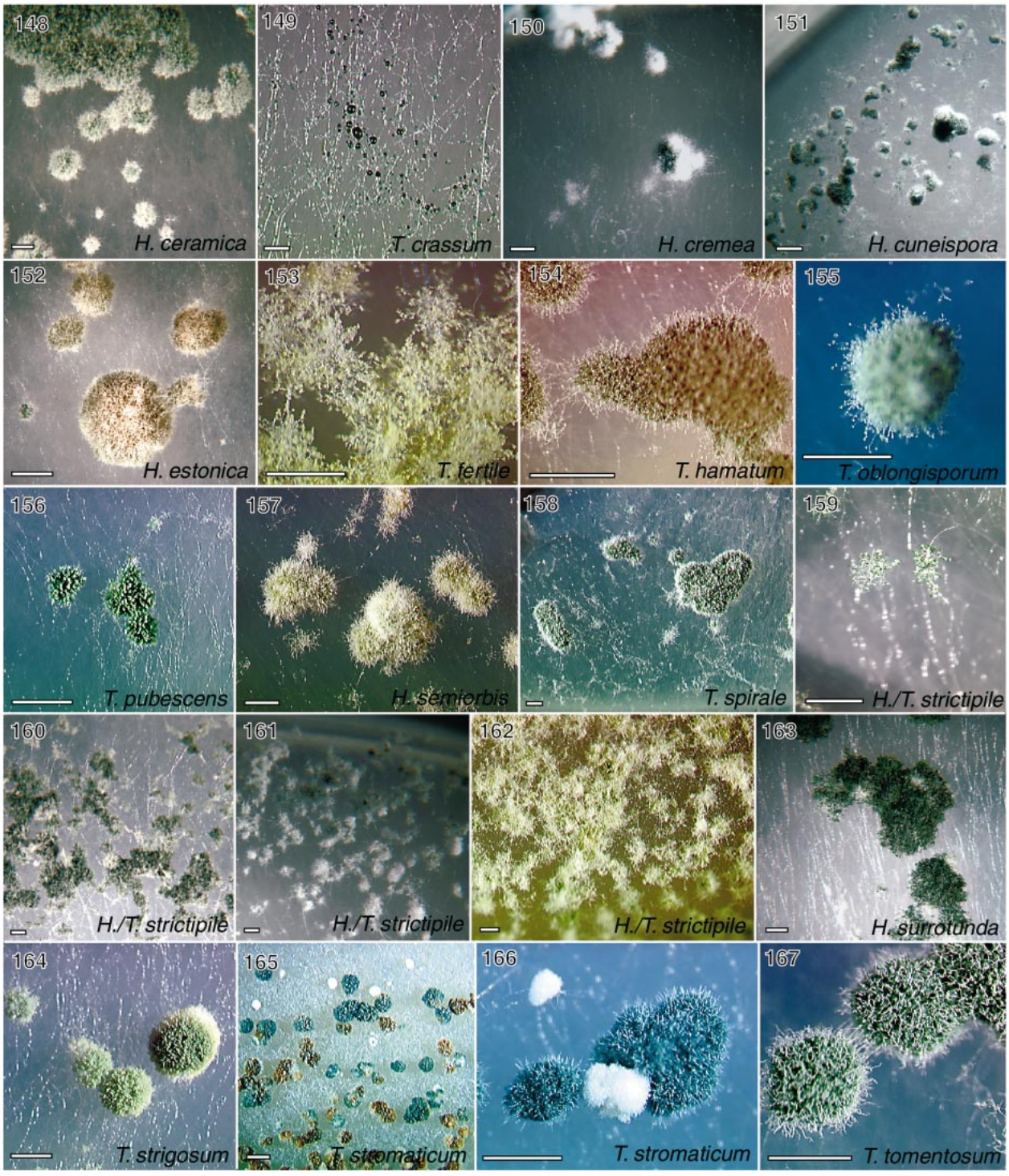
Known distribution.—Canada (Ontario), known only from the type.



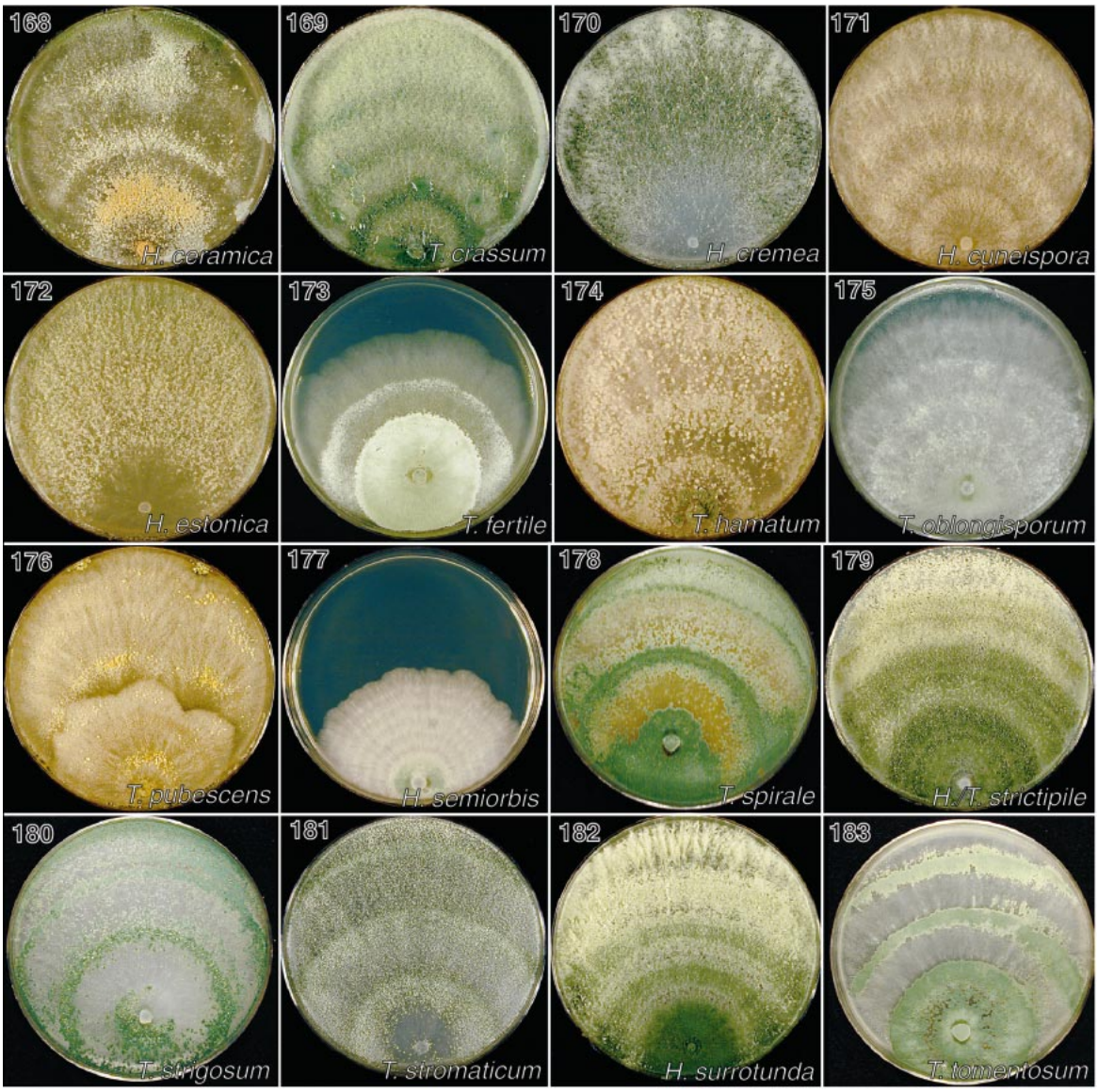
FIGS. 140–147. Stromata. 140. *Hypocrea ceramica* (G.J.S. 88-70). 141. *H. cremea* (holotype). 142. *H. cuneispora* (holotype). 143. *H. estonica* (holotype). 144. *H. semiorbis* (G.J.S. 99-108). 145. *H. surrotunda* (holotype). 146. *H. strictipilosa* (G.J.S. 96-189). 147. *H. strictipilosa* (holotype). Scale bars = 500 μm.



FIGS. 133–139. *Trichoderma tomentosum* (DAOM 178713a, ex-type). 133–135. Conidiophores and elongations. 136, 137. Verticillium- to gliocladium-like synanamorph. 138. Phialides and conidia. 139. Conidia. Scale bars: 133–135, 136, 138 = 10 μm, 137 = 20 μm, 139 = 5 μm.



FIGS. 148–167. Pustules on CMD at 20 C after 1–2 wk of growth in 12 h darkness/12 h light intervals. 148. *H. ceramica*. 149. *T. crassum* gliocladium-like synanamorph. 150. *H. cremea*. 151. *H. cuneispora*. 152. *H. estonica*. 153. *T. fertile*. 154. *T. hamatum*. 155. *T. oblongisporum*. 156. *T. pubescens*. 157. *H. semiorbis*. 158. *T. spirale*. 159–162. *H. strictipilosa*/*T. strictipile*. 163. *H. surrotunda*. 164. *T. strigosum*. 165, 166. *T. stromaticum*. 167. *T. tomentosum*. 148–154, 156, 158, 163, 164, 167 = type isolates, 155 = DAOM 167085, 157 = DAOM 167636, 159 = G.J.S. 98-113, 160 = G.J.S. 98-110, 161 = G.J.S. 94-97, 162 = DAOM 167646 (ex-type isolate of *T. fasciculatum*), 165 = G.J.S. 00-91, 166 = G.J.S. 00-141. Scale bars = ca 1 mm.



FIGS. 168–183. Colonies on PDA at 25 C after 1–2 wk of growth in 12 h darkness/12 h light intervals. 168. *H. ceramica*. 169. *T. crassum*. 170. *H. cremea*. 171. *H. cuneispora*. 172. *H. estonica*. 173. *T. fertile*. 174. *T. hamatum*. 175. *T. oblongisporum*. 176. *T. pubescens*. 177. *H. semiorbis*. 178. *T. spirale*. 179. *T. strictipile*. 180. *T. strigosum*. 181. *T. stromaticum*. 182. *H. surrotunda*. 183. *T. tomentosum*. 168 = G.J.S. 88-70, 169–176, 178–180, 182, 183 = ex-type isolates, 177 = G.J.S. 81-195, 181 = G.J.S. 00-91.

Culture examined.—CANADA. ONTARIO: South March, under *Ulmus* sp. bark, 30 Oct 1980, N. Grainger, H. L. Dickinson (DAOM 178713a, ex-type).

ACKNOWLEDGMENTS

We appreciate Dr. John Bissett and Ms. Carolyn Babcock (DAOM) for providing us with cultures from the DAOM collection. Dr. Steven Rehner kindly provided an EF1-α primer sequence and Andrew N. Miller gave helpful advice on the use of RPB2. We also thank Drs. Elwin L. Stewart, David M. Geiser and Amy Y. Rossman for their comments on the paper. This study was supported in part by the United States National Science Foundation (PEET) grant 9712308, "Monographic Studies of Hypocrealean Fungi: *Hypocrea* and *Hypomyces*" to Pennsylvania State University, Department of Plant Pathology. An interactive key to identification of *Trichoderma* species is available at http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm or it can be accessed through the Systematic Botany and Mycology Lab Website, http://nt.ars-grin.gov

LITERATURE CITED

Bissett J. 1991a. A revision of the genus *Trichoderma*. II. Infrageneric classification. Can J Bot 69:2357–2372.

———. 1991b. A revision of the genus *Trichoderma*. III. Section *Pachybasium*. Can J Bot 69:2373–2417.

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556.

Chaverri P, Samuels GJ, Stewart EL. 2001. *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. Mycologia 93:1113–1124.

Cunningham CW. 1997. Is incongruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Syst Biol 46:464–478.

Dodd SL, Crowhurst RN, Rodrigo AG, Samuels GJ, Hill RA, Stewart A. 2000. Examination of *Trichoderma* phylogenies derived from ribosomal DNA sequence data. Mycol Res 104:23–34.

Doi Y. 1966. A revision of Hypocreales with cultural observations. I. Some Japanese species of *Hypocrea* and *Podostroma*. Bull Natl Sci Mus 9:345–357.

———. 1972. Revision of the Hypocreales with cultural observations. IV. The genus *Hypocrea* and its allies in Japan (2). Enumeration of the species. Bull Natl Sci Mus 15:649–751.

Ellis JB, Everhart BM. 1892. The North American Pyrenomycetes. Newfield, New Jersey: Ellis & Everhart. 793 p.

Gams W, Bissett J. 1998. Morphology and identification of *Trichoderma*. In: Kubicek C, Harman G, eds. *Trichoderma* and *Gliocladium*. Bristol, Pennsylvania: Taylor & Francis Inc. p 3–34.

Huelsenbeck JP, Bull JJ, Cunningham CW. 1996. Combining data in phylogenetic analysis. Trends Ecol Evol 11:152–158.

Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA cluster. Fungal Genet Biol 24:298–309.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Methuen London Ltd. 252 p.

Kuhls K, Lieckfeldt E, Samuels GJ, Börner T, Meyer W, Kubicek CP. 1997. Revision of *Trichoderma* sect. *Longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. Mycologia 89:442–460.

Lieckfeldt E, Kuhls K, Muthumeenakshi S. 1998a. Molecular taxonomy of *Trichoderma* and *Gliocladium* and their teleomorphs. In: Kubicek C, Harman G, eds. *Trichoderma*, *Gliocladium*. London: Taylor & Francis Inc. p 35–56.

———, Kullnig C, Kubicek CP, Samuels GJ, Börner T. 2001. *Trichoderma aureoviride*: phylogenetic position and characterization. Mycol Res 105:313–322.

———, Samuels GJ, Börner T, Gams W. 1998b. *Trichoderma koningii*: neotypification and *Hypocrea* teleomorph. Can J Bot 76:1507–1522.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among Ascomycetes: evidence from an RNA Polymerase II Subunit. Mol Biol Evol 16:1799–1808.

Nirenberg HI. 1976. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. Mitt Biol Bundesanst Land-Forstw Berlin-Dahlem 169:1–117.

O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116.

Samuels GJ, Dodd SL, Gams W, Castlebury LA, Petrini O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94:146–168.

———, Pardo-Schultheis R, Hebbar KP, Lumsden RD, Bastos CN, Costa JC, Bezerra JL. 2000. *Trichoderma stromaticum* sp. nov., a parasite of the cacao witches broom pathogen. Mycol Res 104:760–764.

Swofford D. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 24:4876–4882.